

1/60

Fig. 1

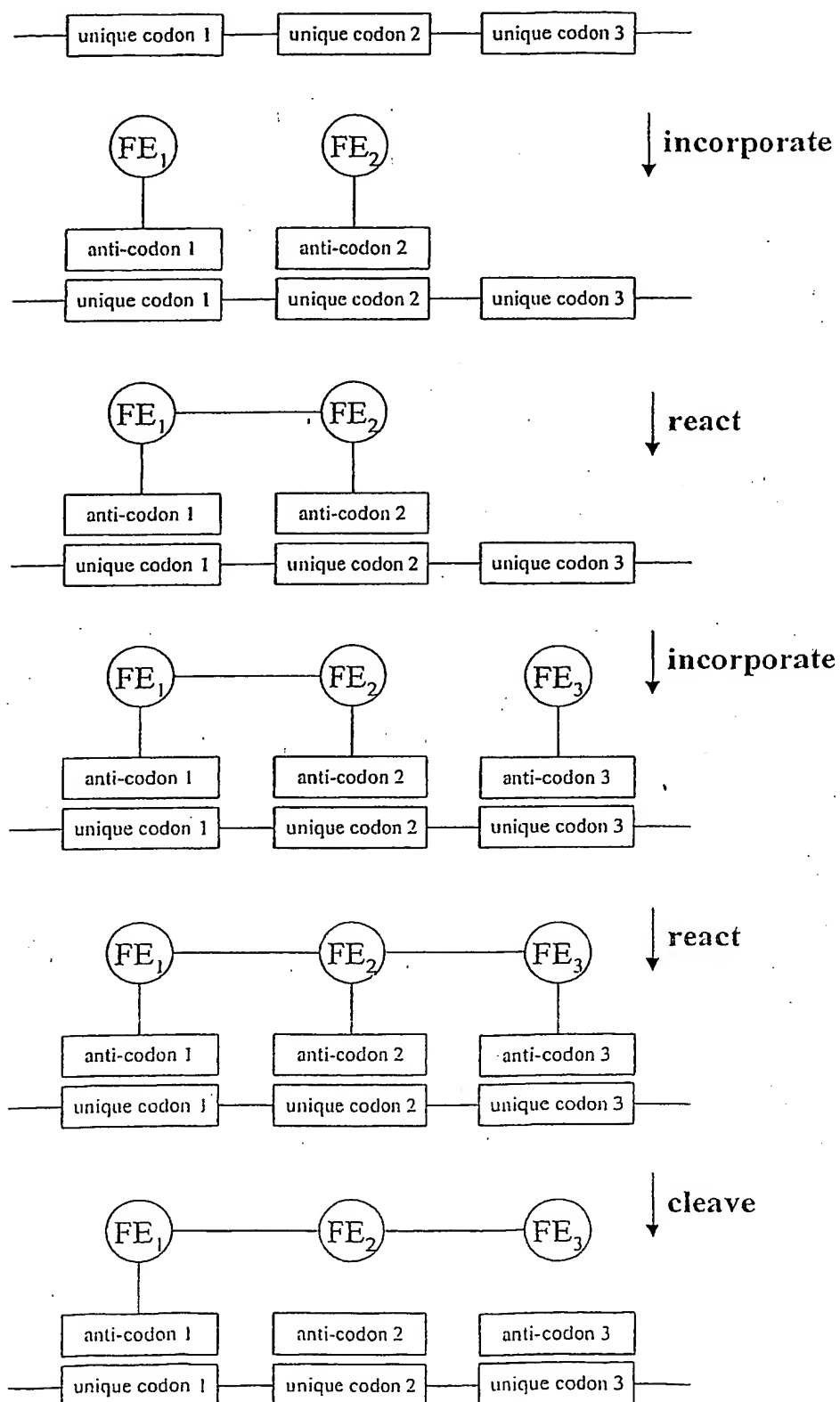


Fig. 2

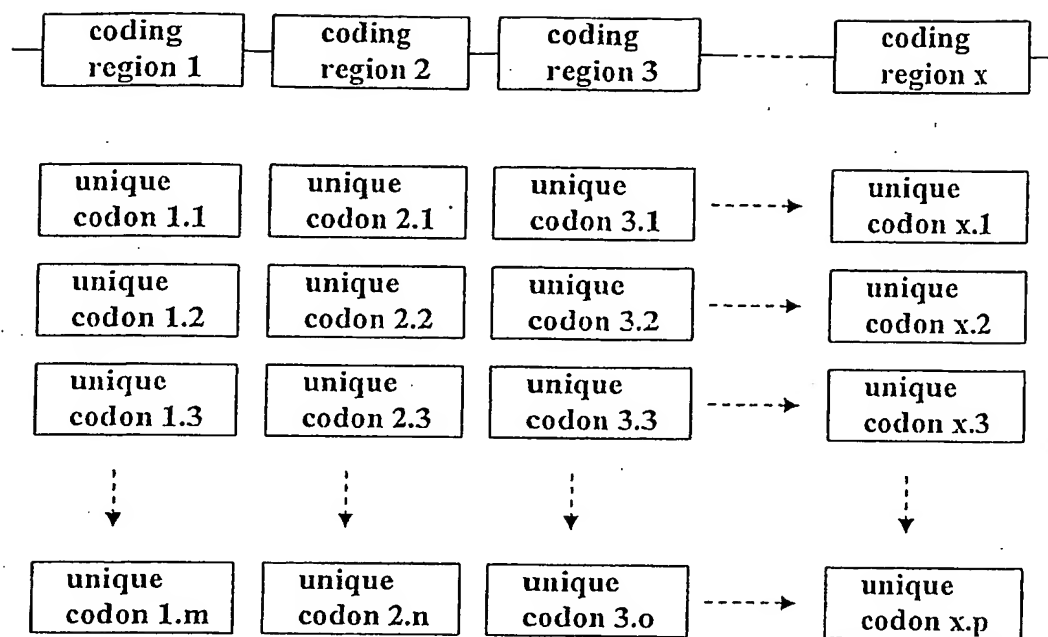
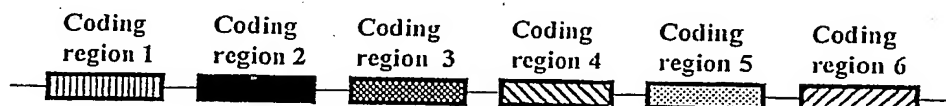


Fig. 3. An oligonucleotide-based building block. Example of coding region design, allowing for high building block diversity.

A.



Coding region	sequence	number of unique codons
1	XXXXXATATTTXXXXX	1024
2	XXXATTTTAXXXXXXX	1024
3	XTAATTTXXXXXXXXXX	1024
4	XXATXXATXXATXXXX	1024
5	GCCCGATTAAAXXCCG	4
6	XAXAXTTXTTXXXGGG	128

X = G or C

B.

Codon 1 GCGCGATATTTGGGCC
 Anti-codon 1 CGCGCTATAAACCCGG

Codon 6 GAGAGTTCTTCGCGGG
 Anti-codon 6 CTCTCAAGAAGCGCCC

Fig. 4. Building blocks.

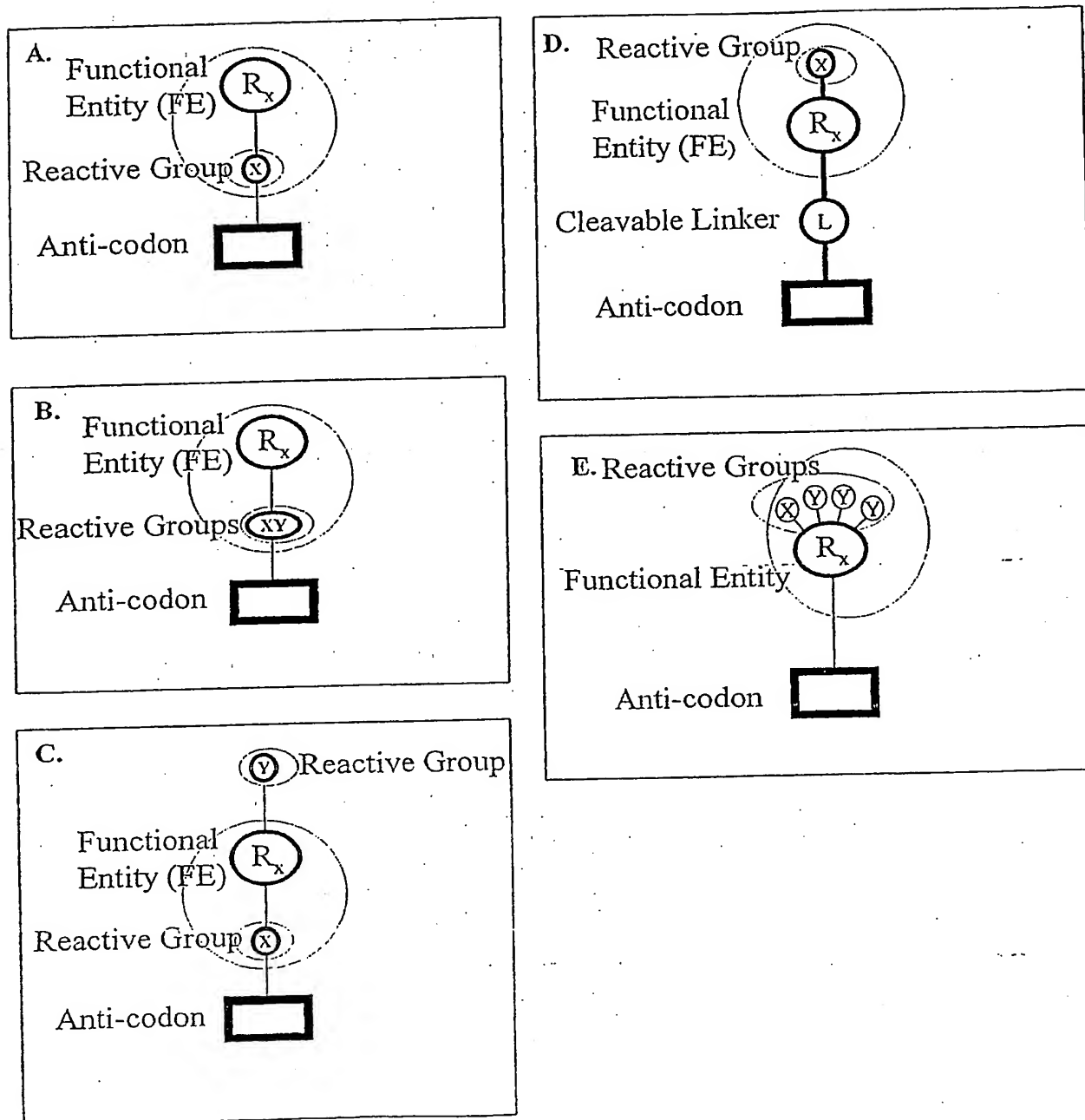


Fig. 5. Exemplary monomer Building Blocks.

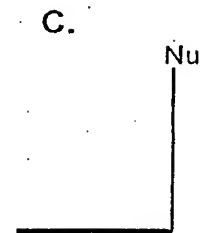
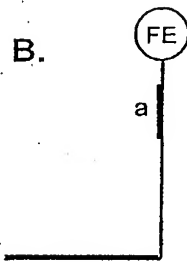
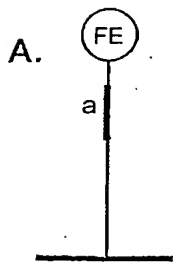
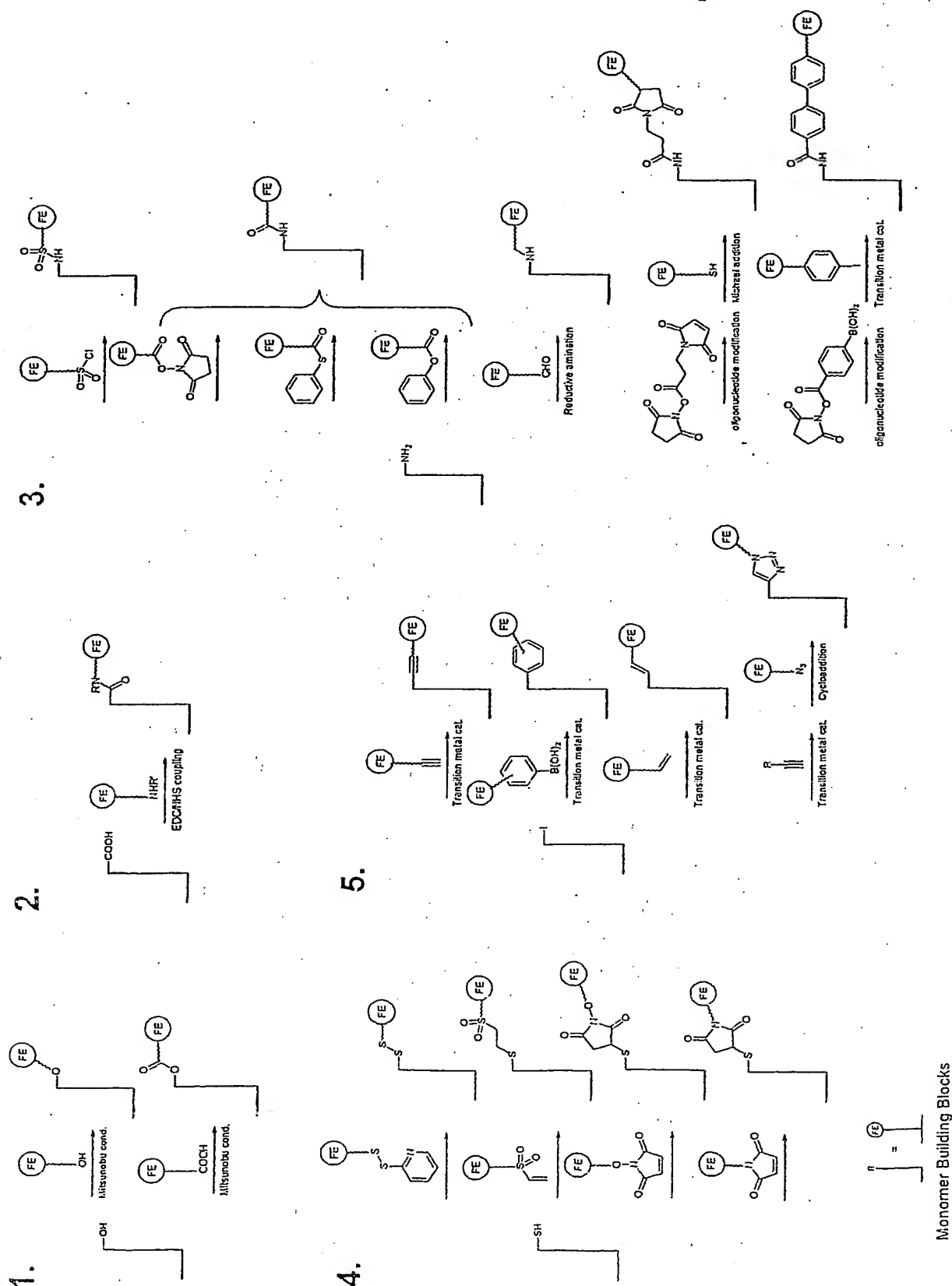


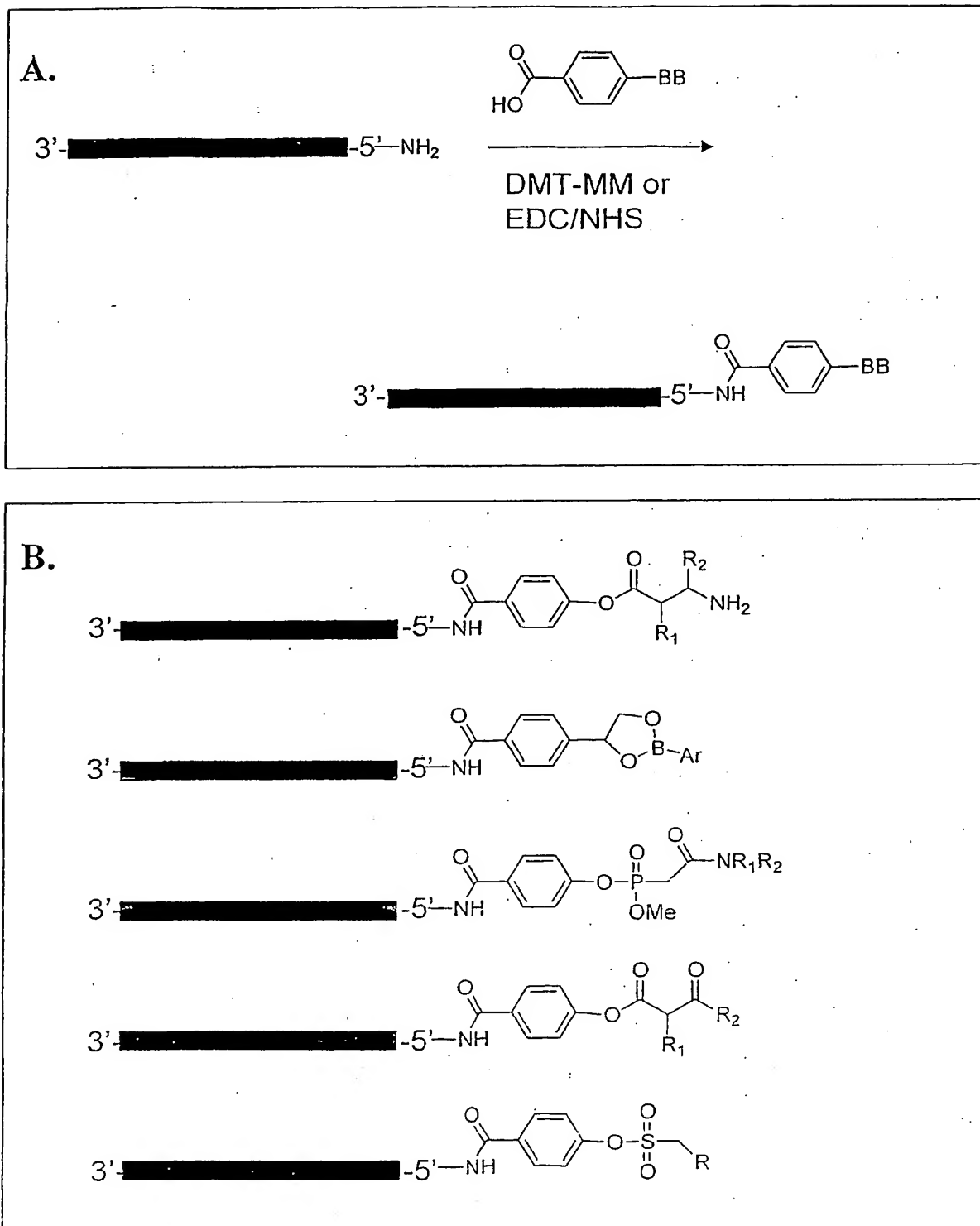
Fig. 6. Preparation of Building Blocks. General examples



Monomer Building Blocks

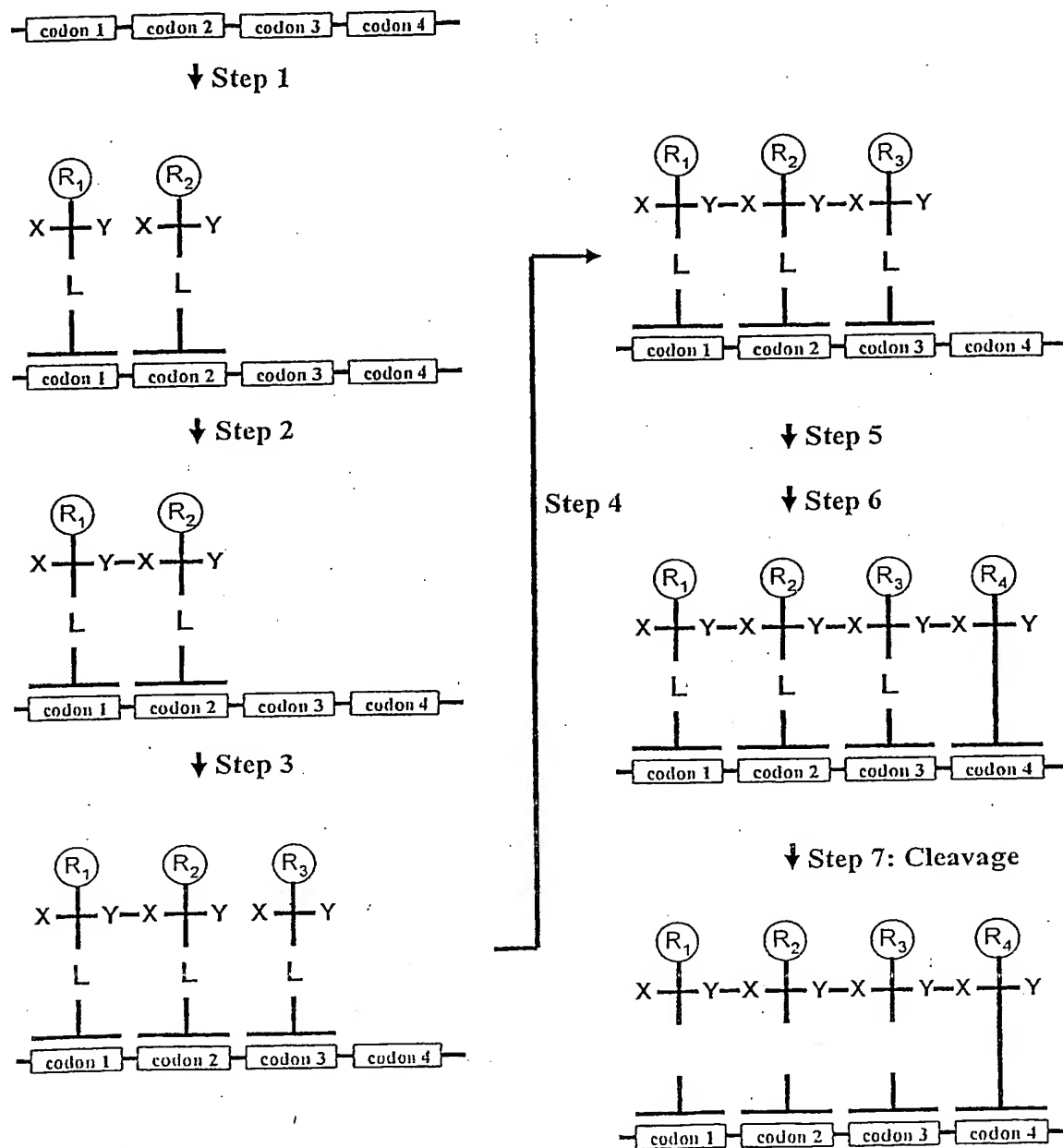
7/60

Fig. 7. Design and synthesis of specific building blocks



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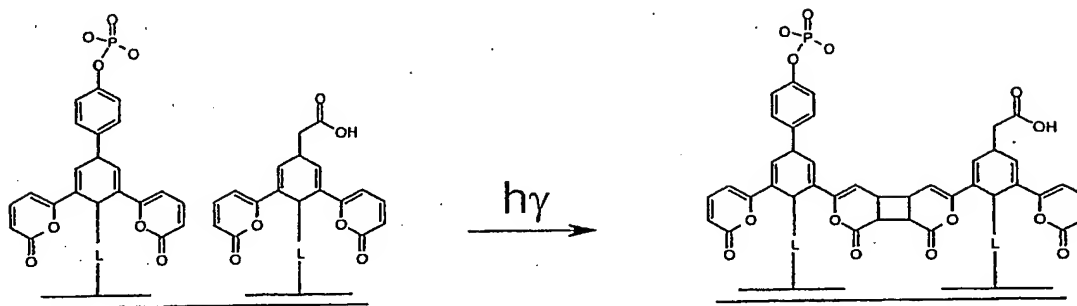
Fig. 8. Templated synthesis of a polymer.



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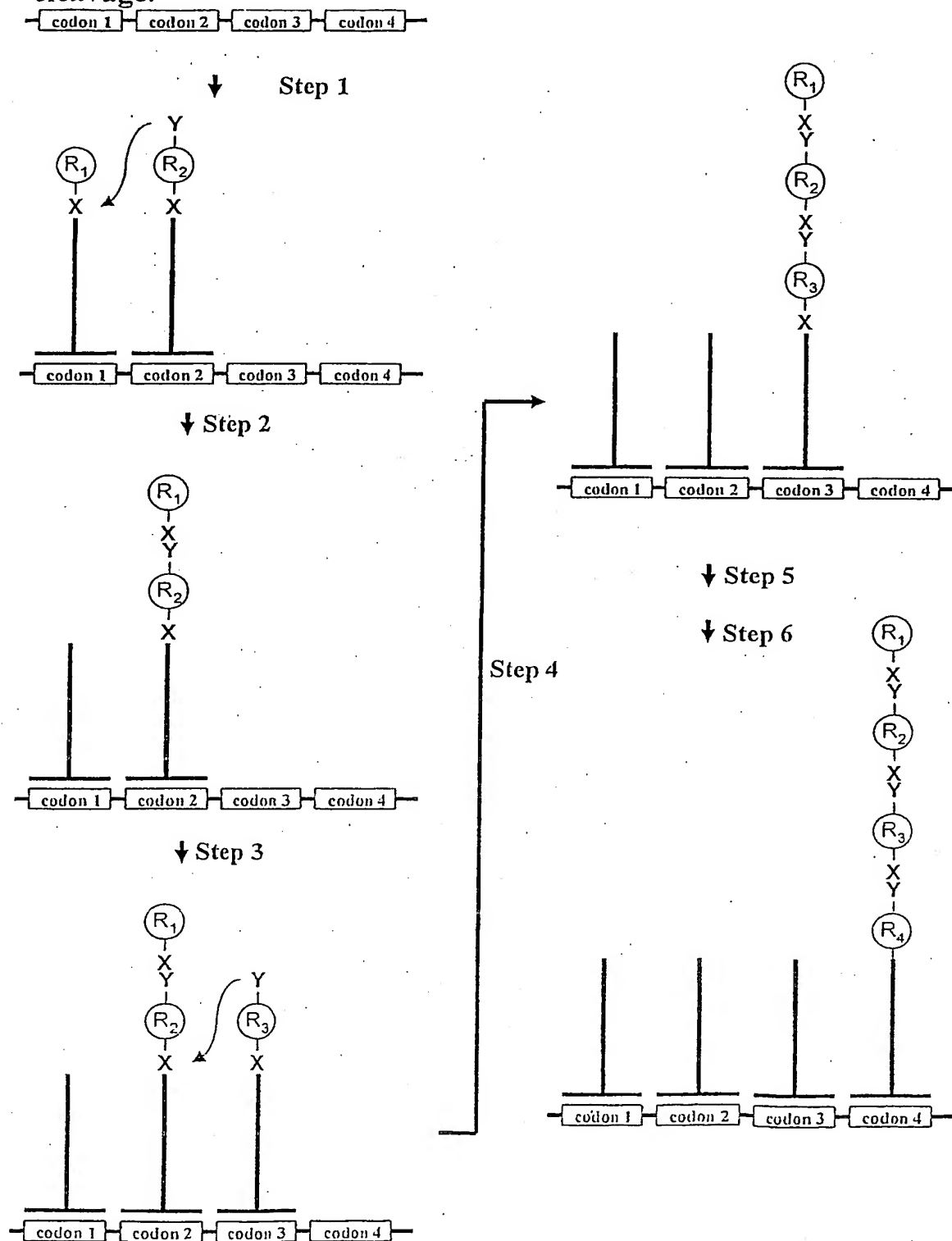
Fig. 8, example 1. Light-induced reaction between symmetric building blocks: Coumarin derivatives.

A



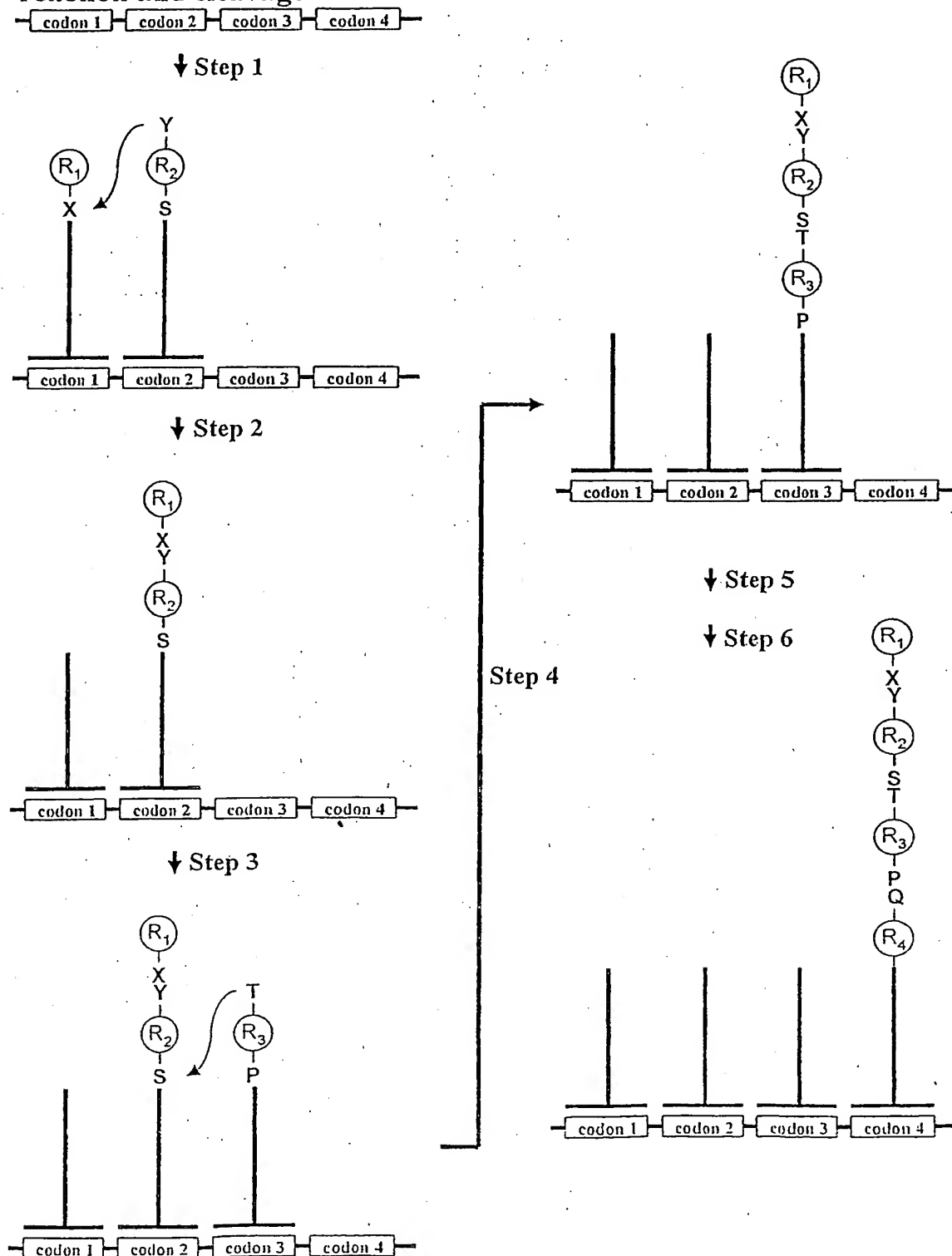
10/60

Fig. 9. Templatd synthesis of a polymer by simultaneous reaction and cleavage.



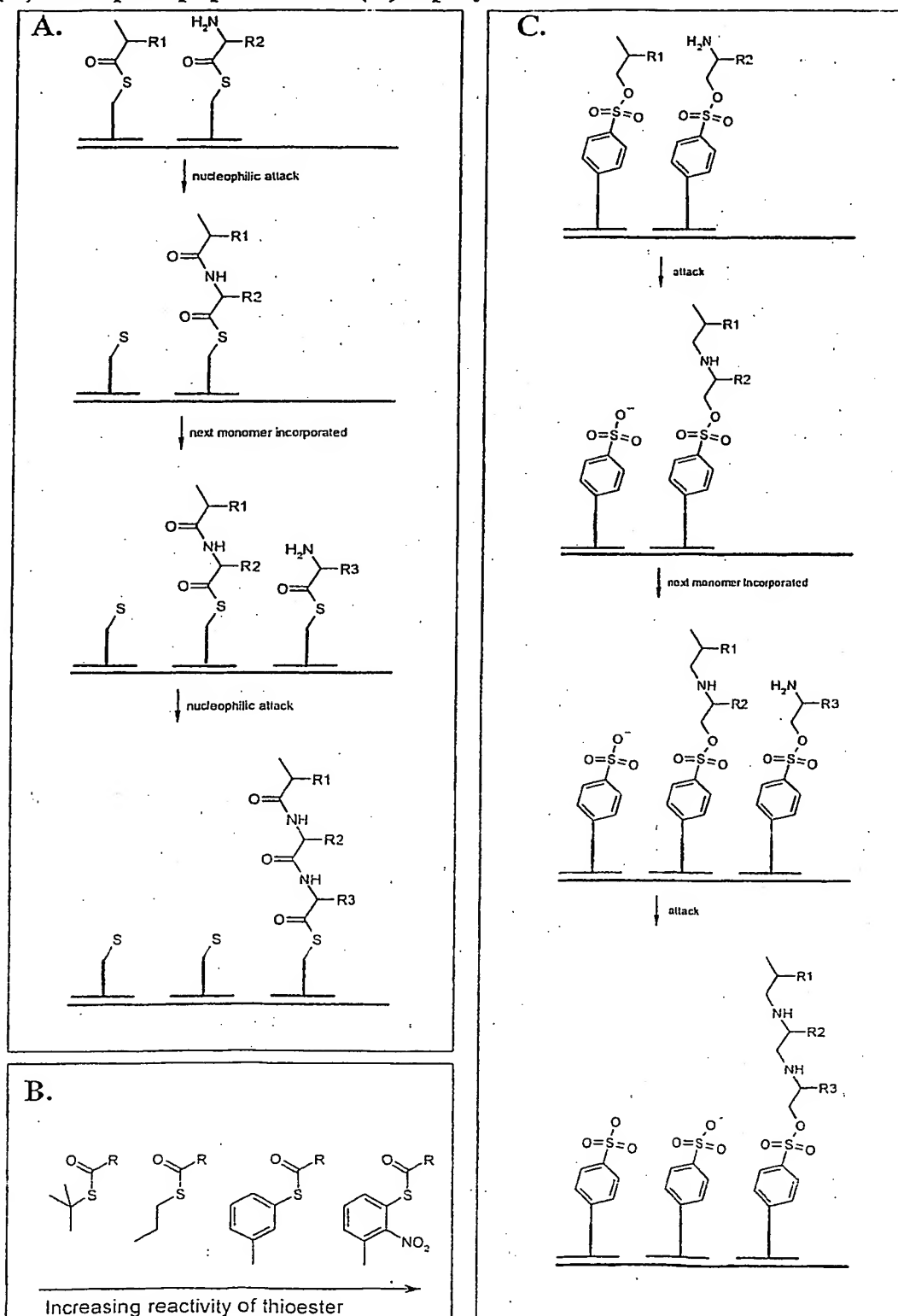
11/60

Fig. 10. Templatd synthesis of a mixed polymer by simultaneous reaction and cleavage.



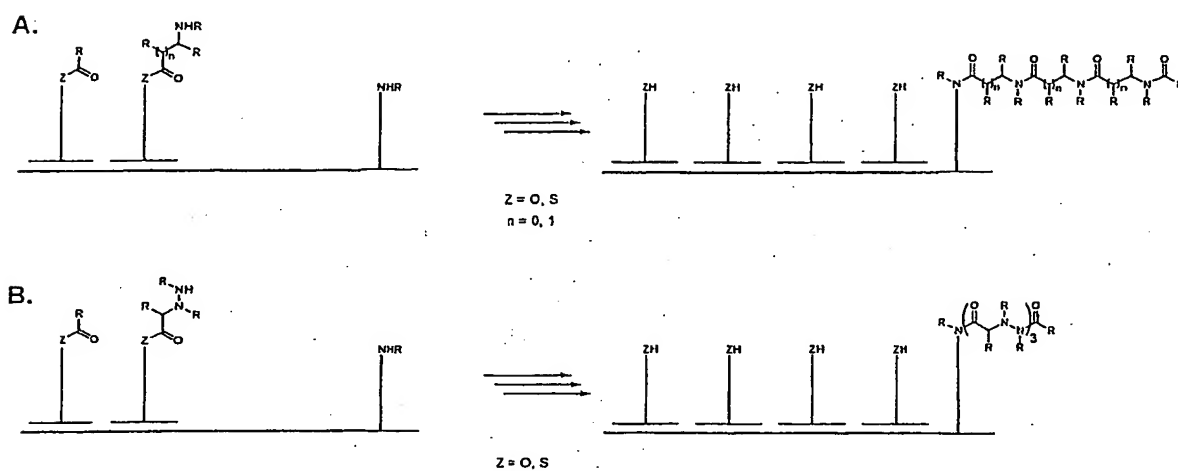
12/60

Fig. 10, example 1. Simultaneous reaction and cleavage: Formation of (A) an alpha-peptide, and (C) a polyamine.



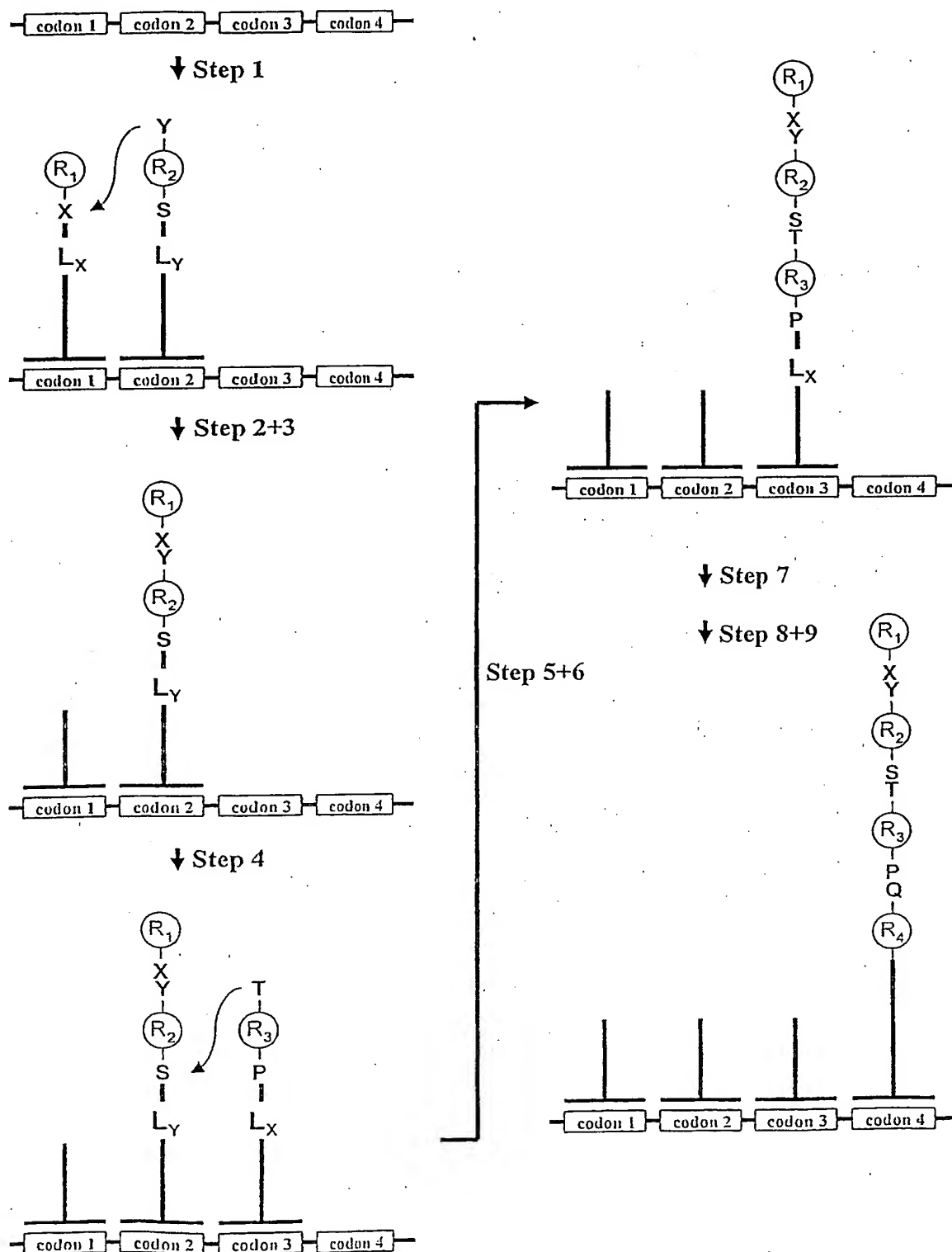
13/60

Fig. 10, example 2. Simultaneous reaction and cleavage: Formation of (A) a peptoid or an alpha- or beta-peptide, and (B) a hydrazino peptide.



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Fig. 11. Templatd synthesis of a polymer, using non-simultaneous reaction and cleavage.



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Fig. 12. Activation of reactive group and release from anti-codon by ring opening.

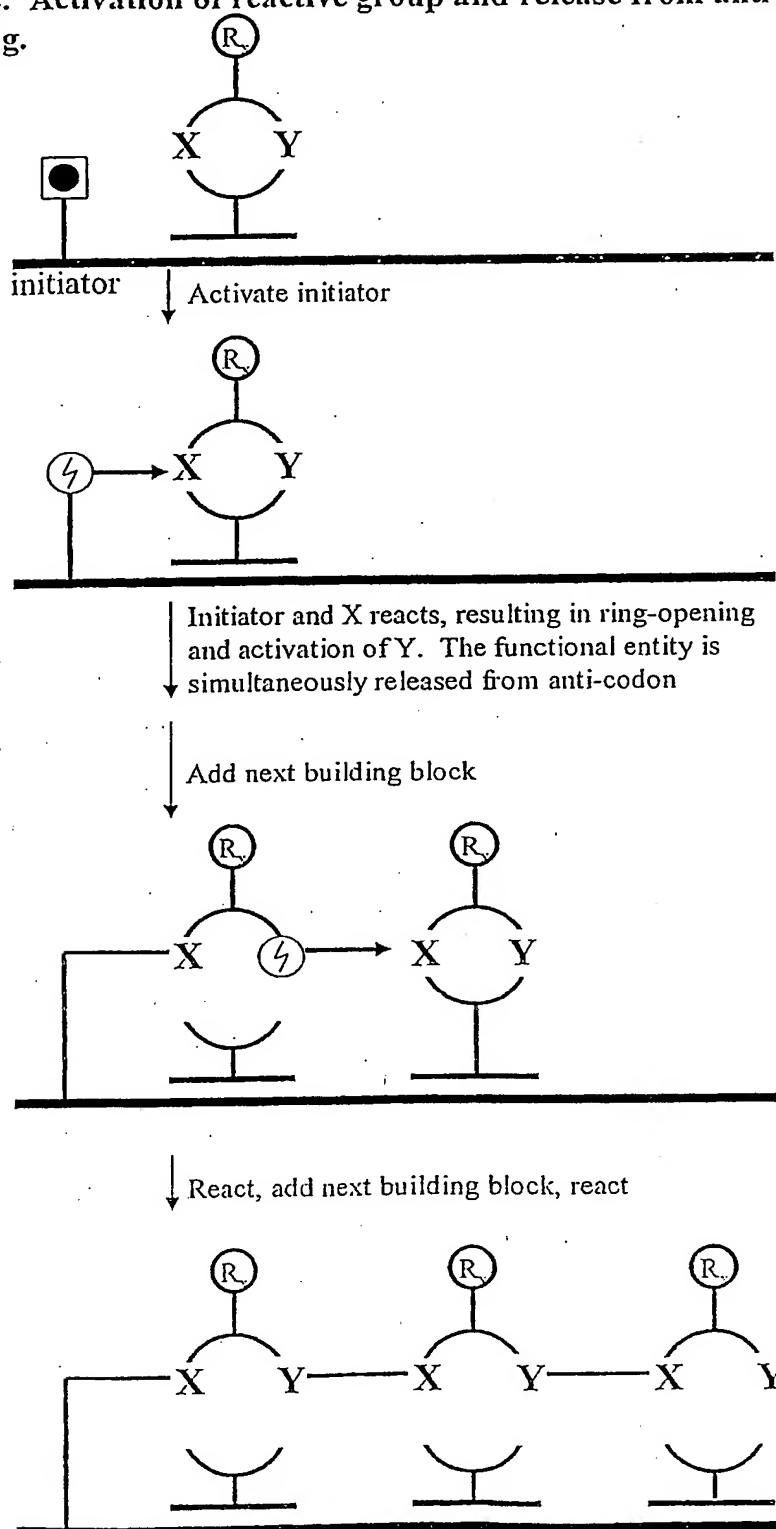


Fig. 13. Symmetric fill-in reaction (symmetric XX building blocks).

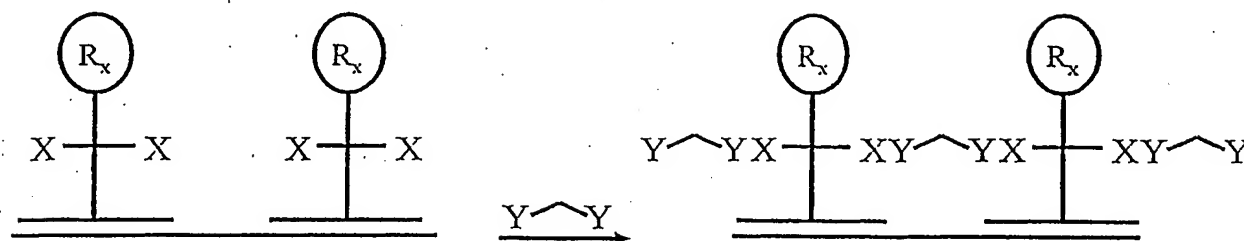
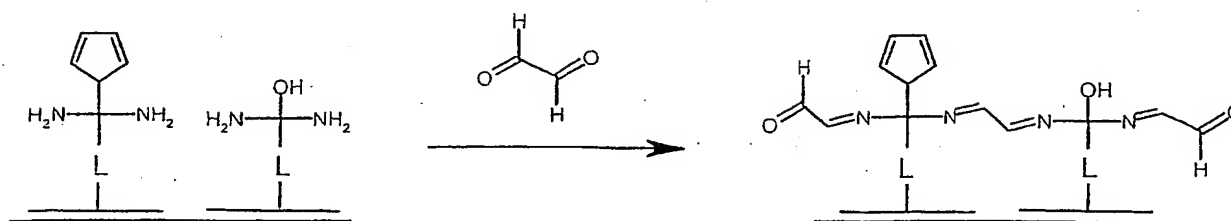


Fig. 13, ex 1. Imine formation by fill-in.



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Fig. 13, example 2. Amide formation.

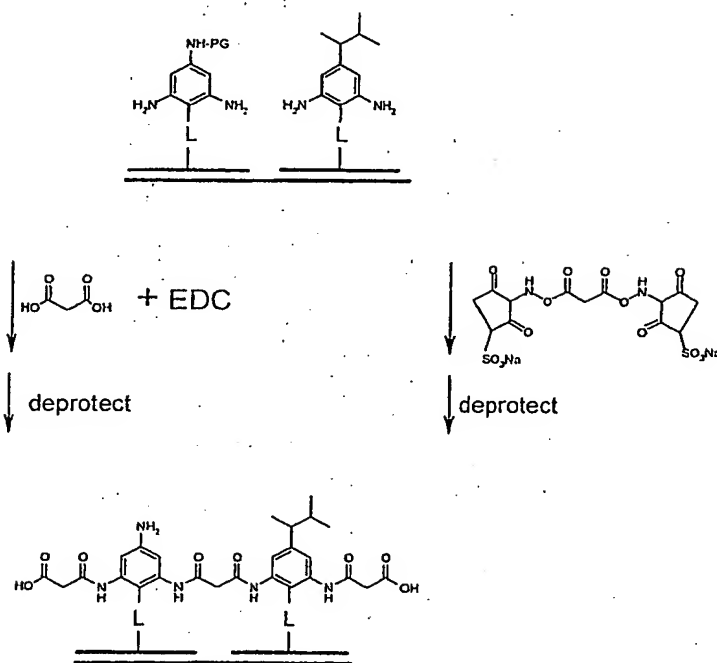
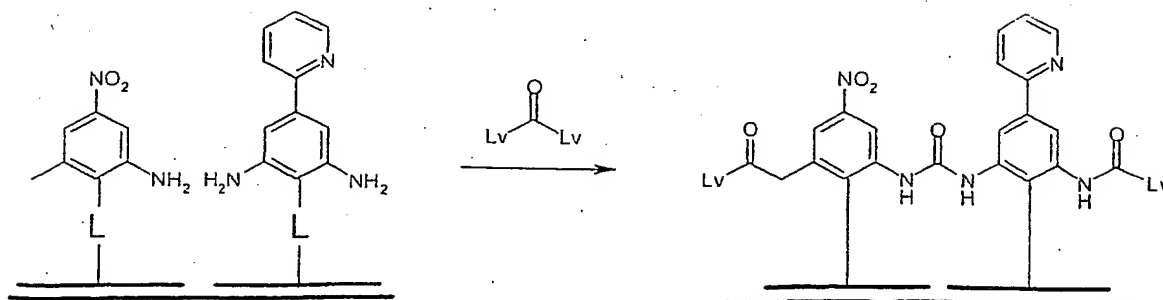


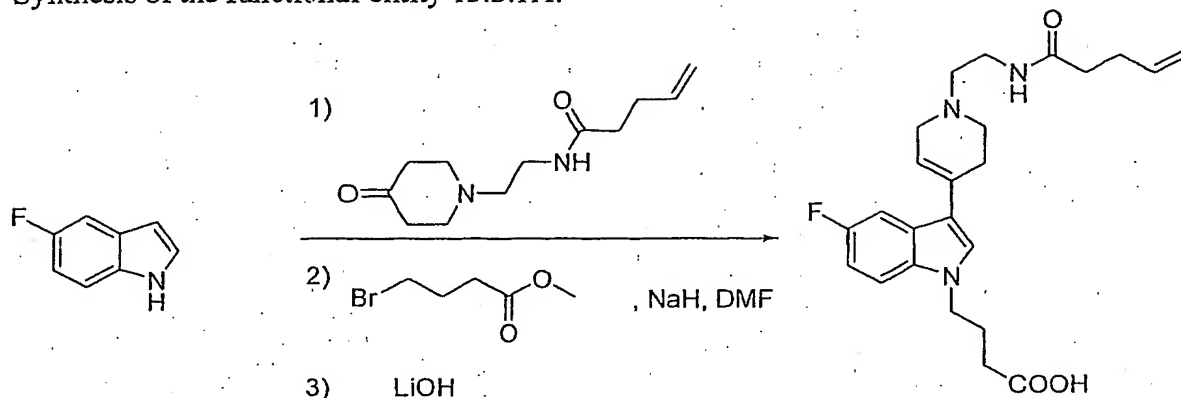
Fig. 13, example 3. Urea formation



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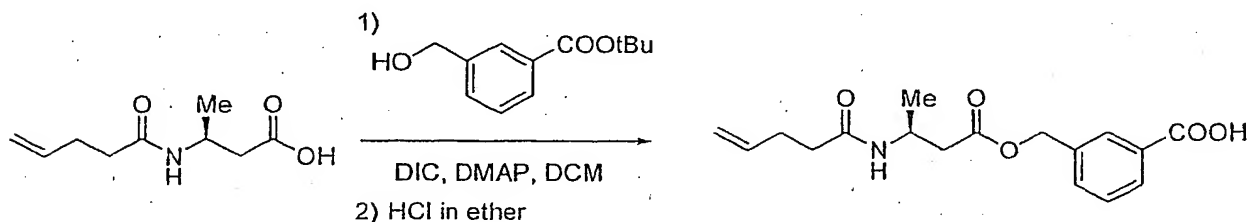
Fig 13, ex 3.1 Urea formation

Synthesis of the functional entity 13.3.1A:



5-Fluoroindole (1 eq) is dissolved in ethanol and treated with pent-4-enoic acid [2-(4-oxopiperidin-1-yl)-ethyl]-amide (1.2 eq) and 2N KOH. The mixture is stirred o/n at reflux. The crude is evaporated and purified by silica gel filtration. The purified material is treated with methyl 3-bromobutanoate (1.2 eq) and NaH (1.5 eq) in DMF at rt. After 5 hours LiOH (10 eq) and water is added and the reaction mixture is stirred at rt o/n. The final product is purified by LC-MS and loaded on a DNA oligo containing an amino function.

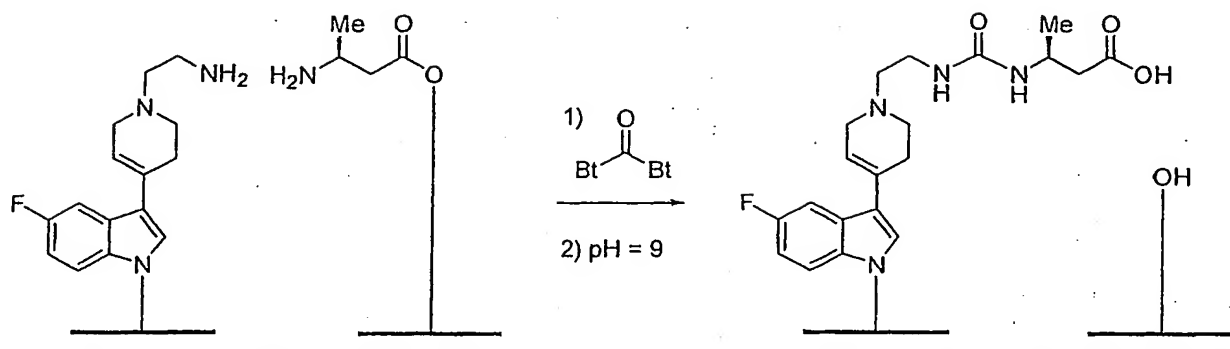
Synthesis of the functional entity 13.3.1B:



3-Pent-4-enoylamino-butanoic acid (1 eq) is treated with 3-hydroxymethyl-benzoic acid tert-butyl ester (1.2 eq), DIC (1.2 eq) and DMAP (0.2 eq) in DCM. The reaction mixture is stirred o/n at rt. The crude is evaporated and purified by silica gel filtration. The purified material is dissolved in diethyl ether and treated with HCl in diethyl ether. After stirring for 3 hours the mixture is evaporated and the crude material loaded on a DNA oligo containing an amino function.

Fill in experiment using functional entity 13.3.1A and 13.3.1B:

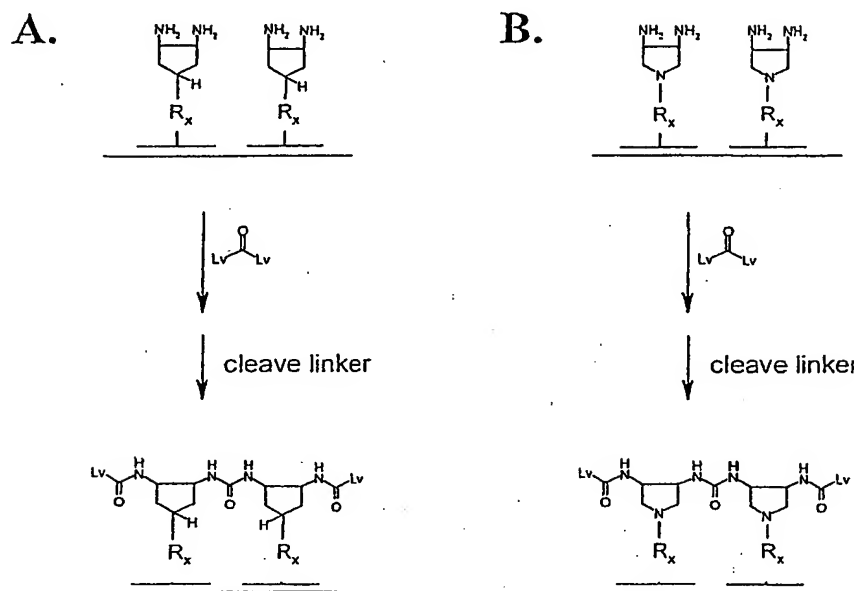
19/60



The two loaded oligos are mixed with a template oligo in in hepes buffer (pH = 7.5) and 100 mM NaCl. 1,1'-Carbonylbisbenzotriazole (0.1M in MeOH) is added and the mixture is left at rt for 4 hours. pH is then adjusted to 9 and the mixture is left at rt o/n.

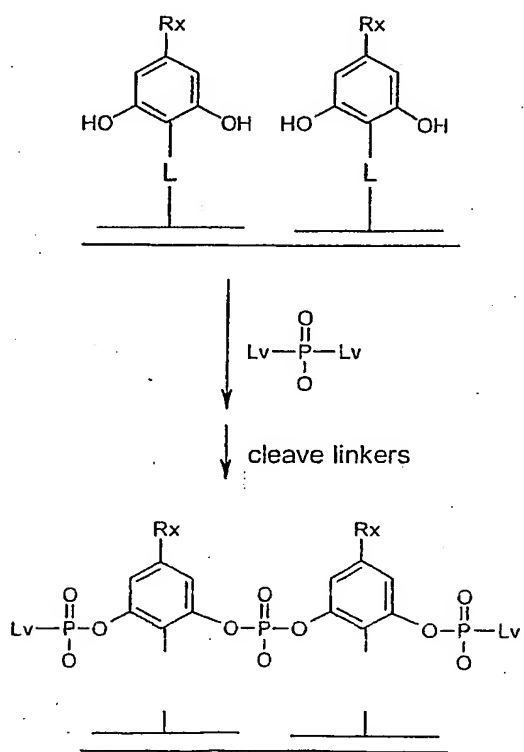
20/60

Fig. 13, ex 4. Chiral and non-chiral templated molecule



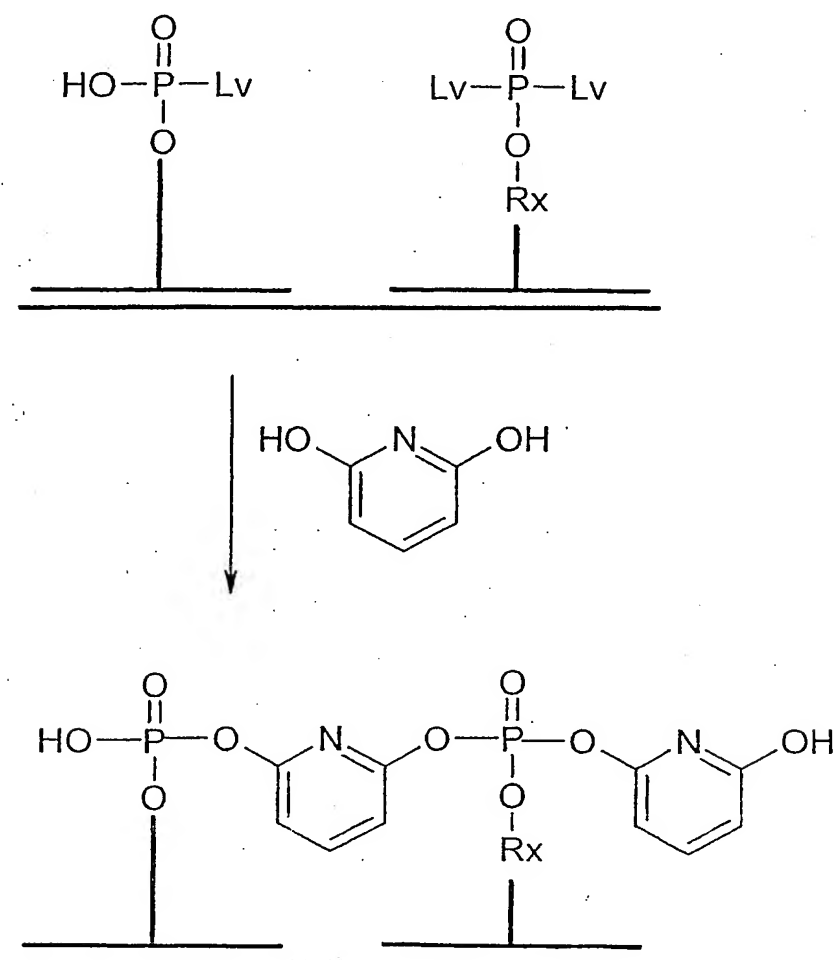
21/60

Fig. 13, ex 5. Symmetric fill-in: Formation of a phosphodiester bond.



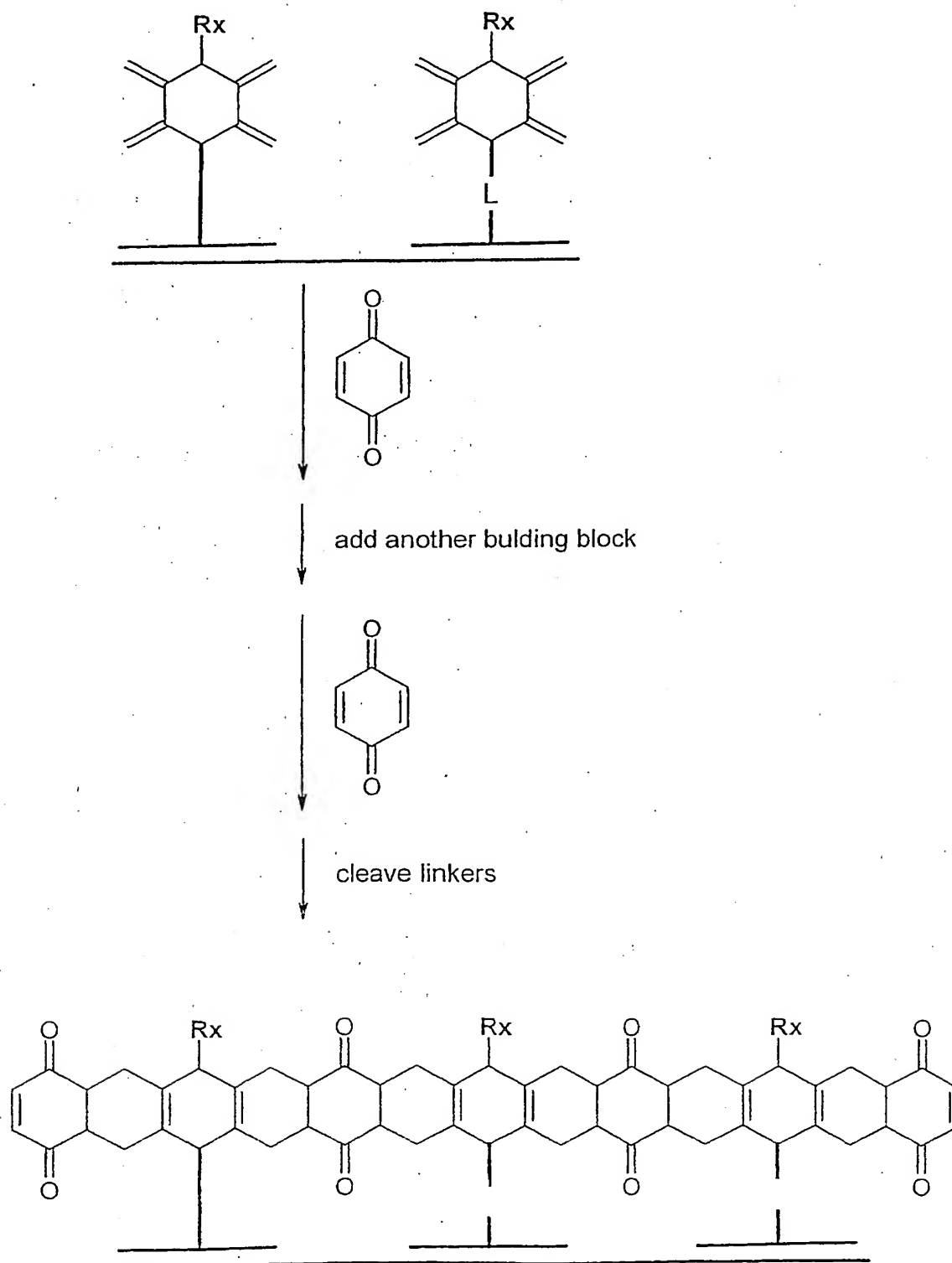
22/60

Fig. 13, ex 6. Fill-in: Phosphodiester formation with one reactive group in each building block



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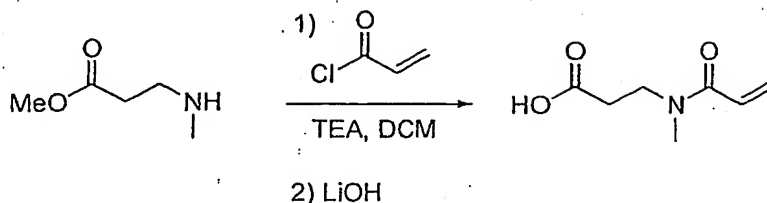
Fig. 13, ex 7. Pericyclic reaction.



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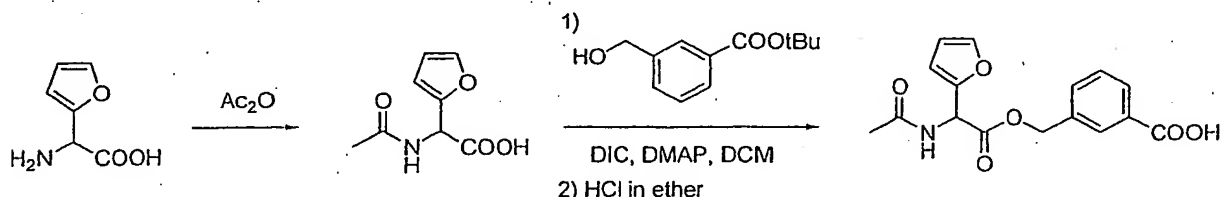
Figure 13, ex 7.1 Pericyclic reaction

Synthesis of the functional entity 13.7.1A:



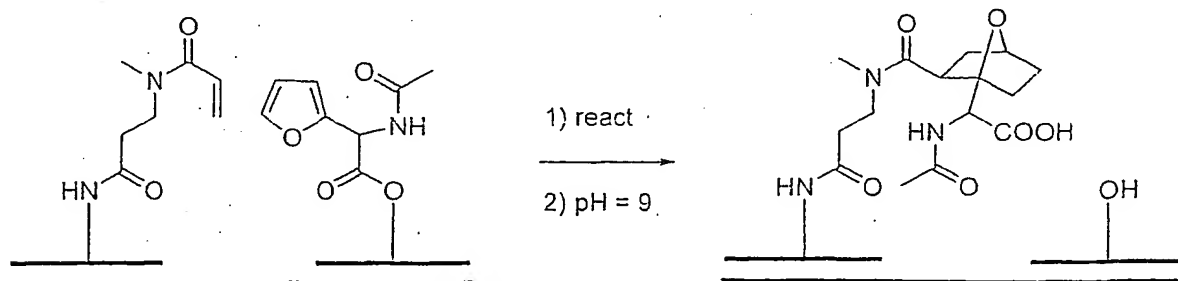
3-Methylamino-propionic acid methyl ester (1 eq) is dissolved in DCM and triethylamine (2 eq). The mixture is cooled to 0° and treated with acryloyl chloride (1.5 eq). After 2 hours the reaction mixture is evaporated, redissolved in THF and treated with LiOH (10 eq) and water. The mixture is left at rt for 3 hours. The crude is extracted with EtOAc (2x). The combined organic phases are dried over MgSO_4 and evaporated. The product is purified by LC-MS and loaded on a DNA oligo containing an amino function.

Synthesis of the functional entity 13.7.1B:



Amino-furan-2-yl-acetic acid (1 eq) is treated with acetic anhydride (3 eq) at rt for 1 hour. The crude is evaporated and the product purified by LC-MS and then treated with 3-hydroxymethyl-benzoic acid tert-butyl ester (1.2 eq), DIC (1.2 eq) and DMAP (0.2 eq) in DCM. The reaction mixture is stirred o/n at rt. The crude is evaporated and purified by silica gel filtration. The purified material is dissolved in diethyl ether and treated with HCl in diethyl ether. After stirring for 3 hours the mixture is evaporated and the crude material loaded on a DNA oligo containing an amino function.

Pericyclic reaction experiment using functional entity 13.7.1A and 13.7.1B:

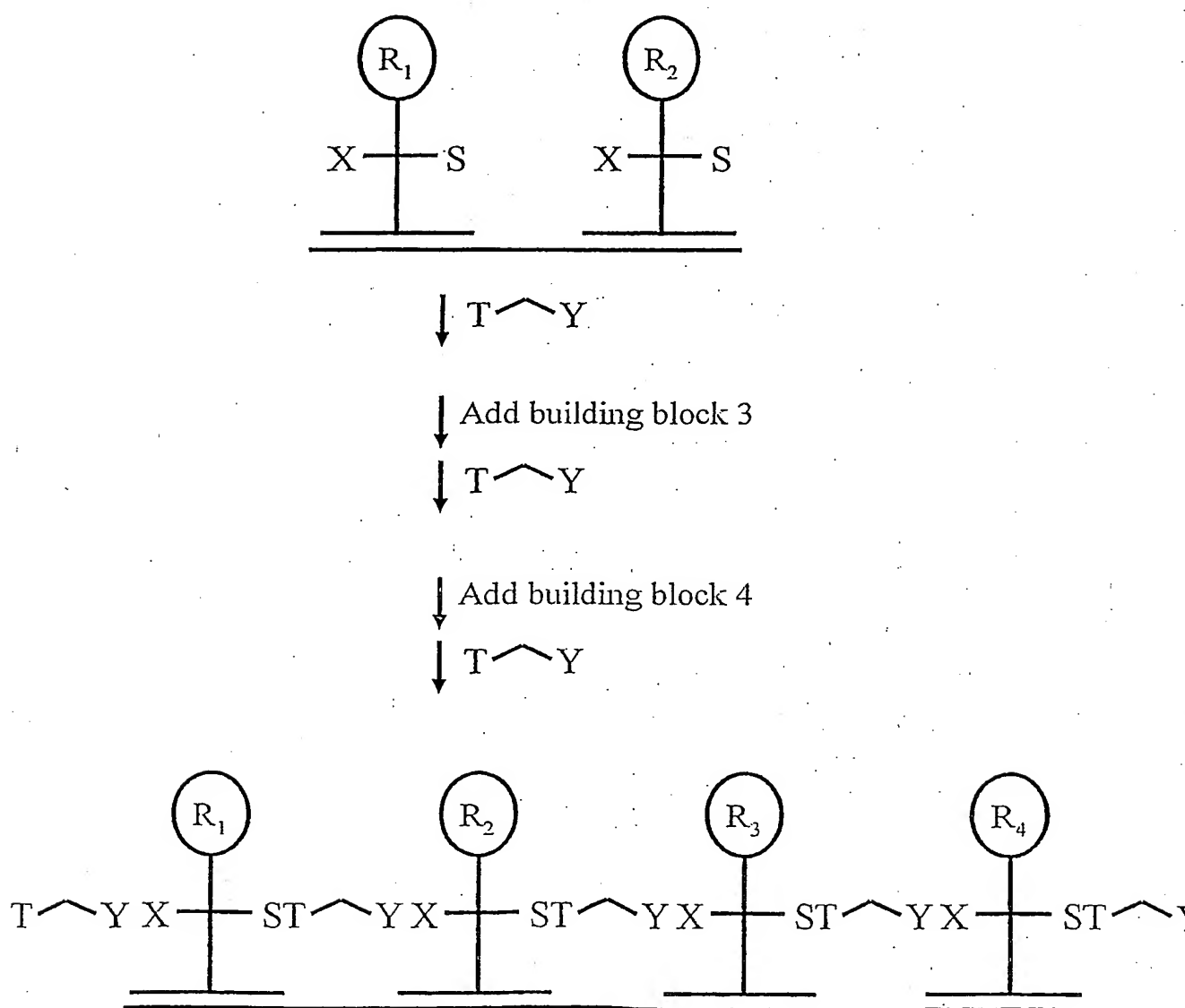


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The two loaded oligos are mixed with a template oligo in in hepes buffer (pH = 7.5) and 100 mM. The mixture is left at rt for 4 hours. pH is then adjusted to 9 and the mixture is left at rt o/n.

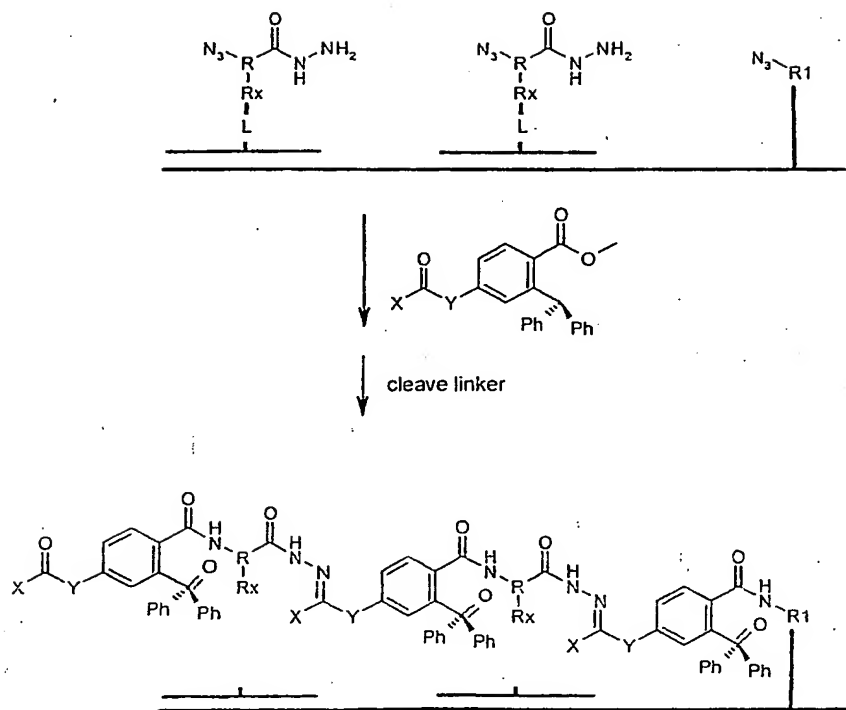
26/60

Fig. 13. "Fill-in" reaction (asymmetric XS monomers).



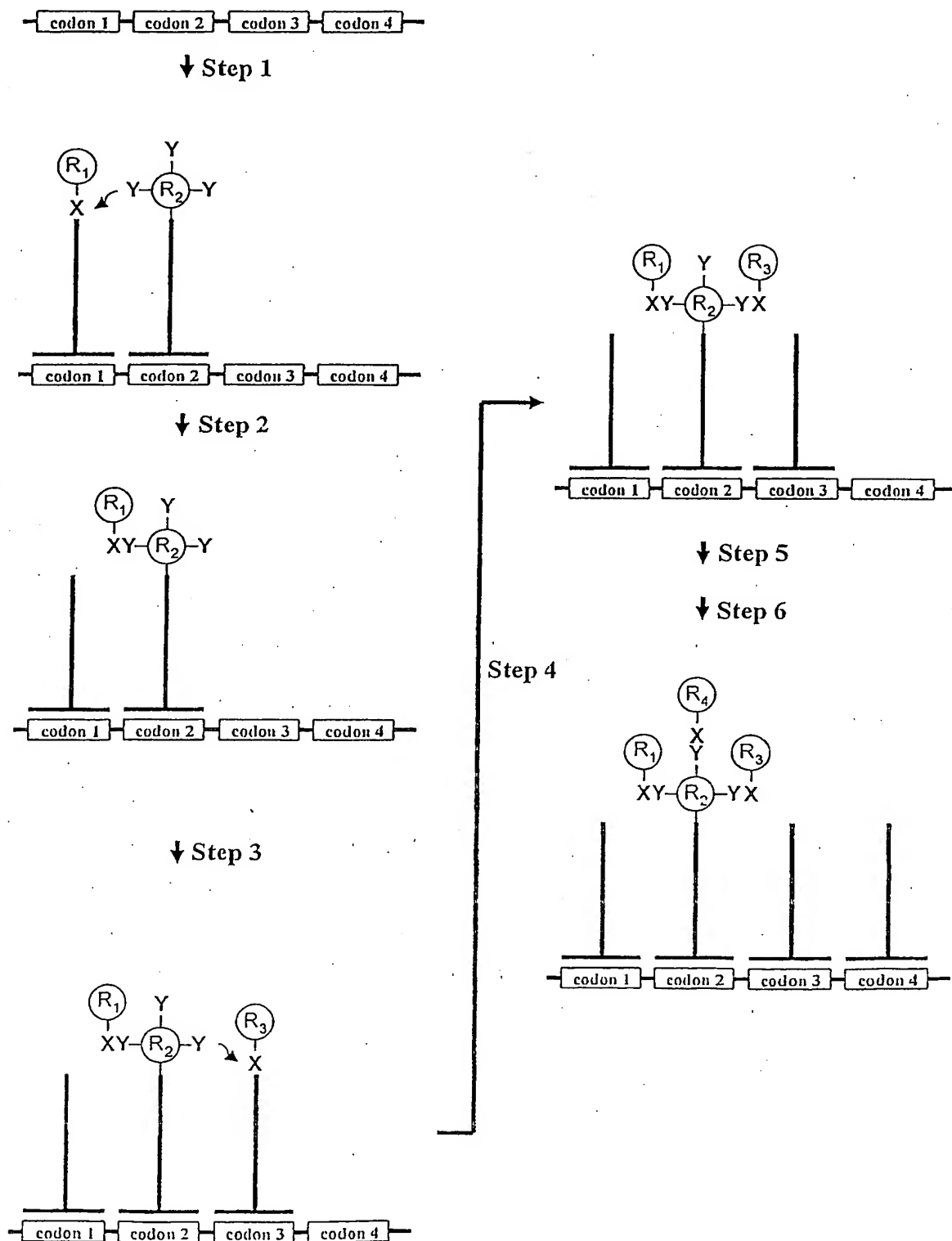
27/60

Fig. 14, example 1.



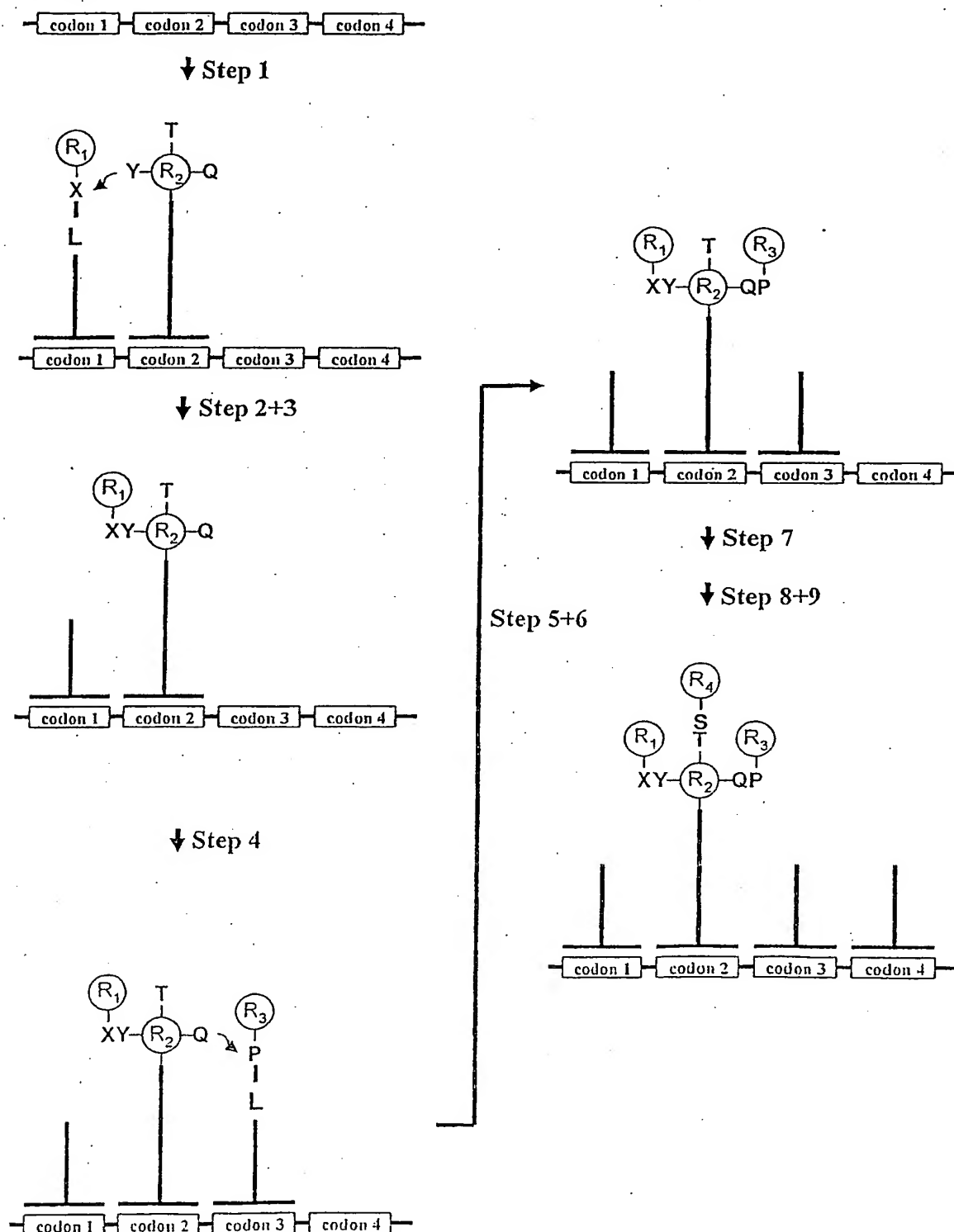
28/60

Fig. 15. Templatd synthesis of a non-linear molecule.



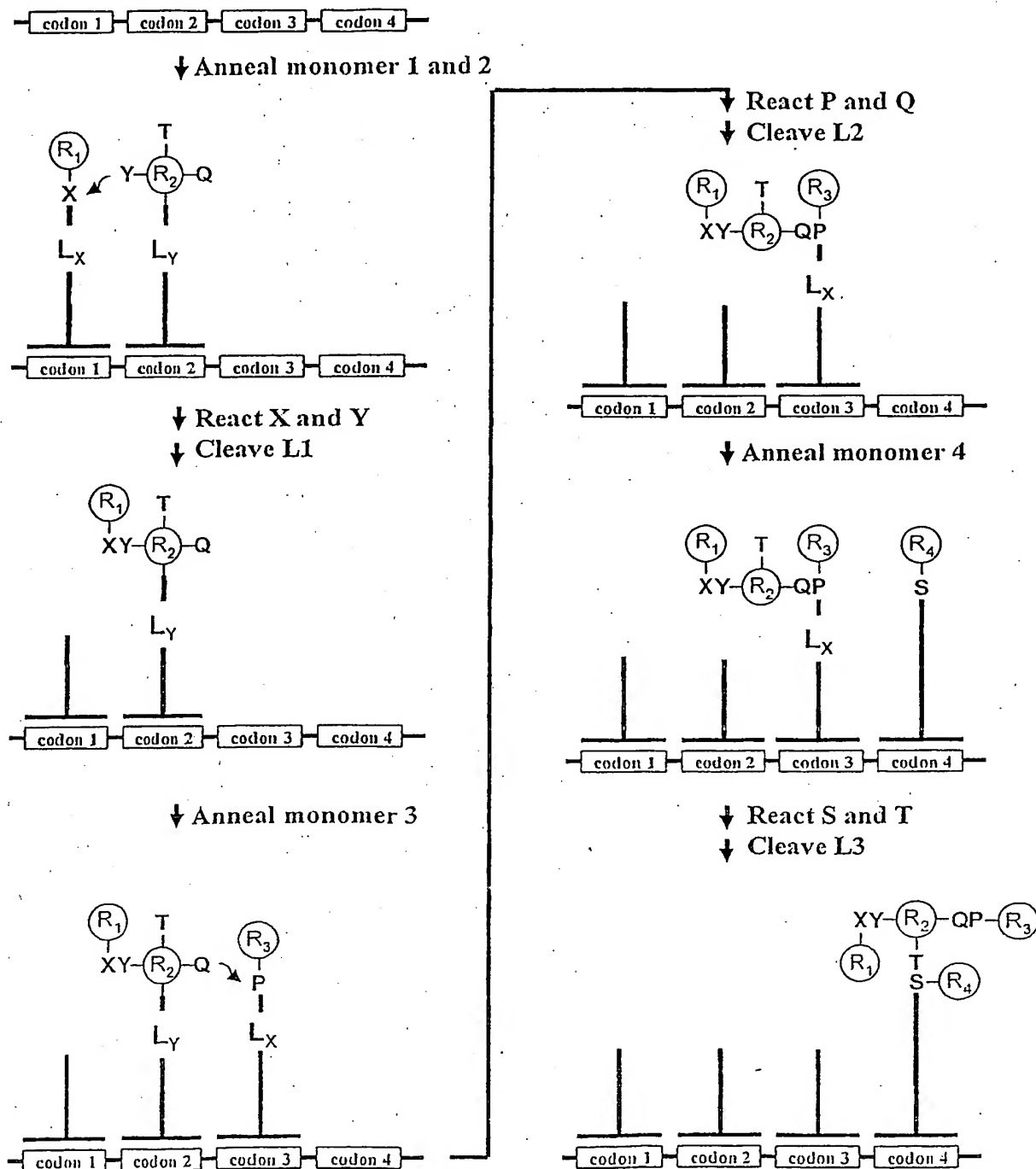
29/60

Fig. 16. Templated synthesis of a non-linear molecule, employing reactive groups of different classes, and non-simultaneous reaction and cleavage.



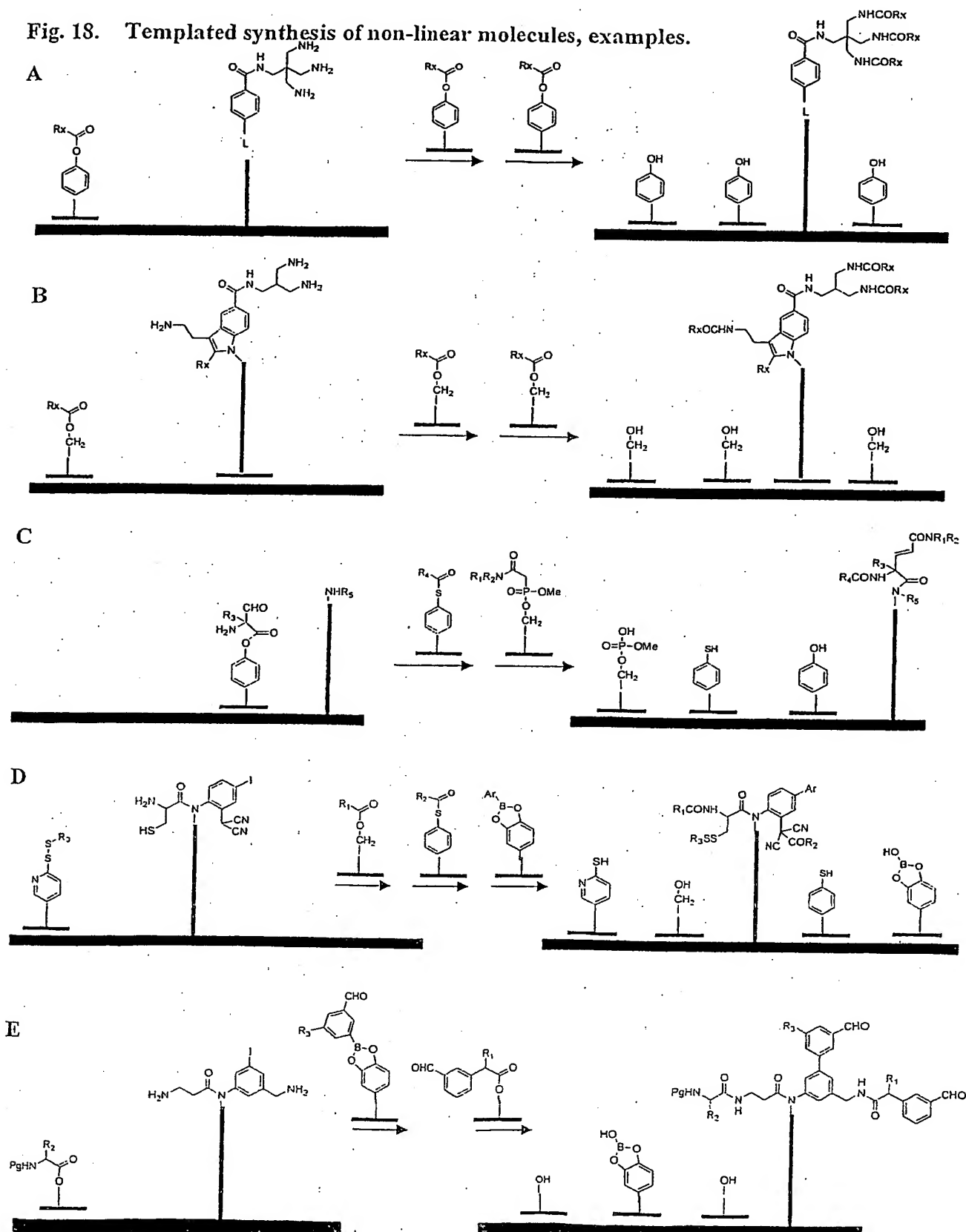
30/60

Fig. 17. Migrating scaffold. Templatd synthesis of a non-linear molecule, by exploiting the increased proximity effect that arises from a "migrating" scaffold.



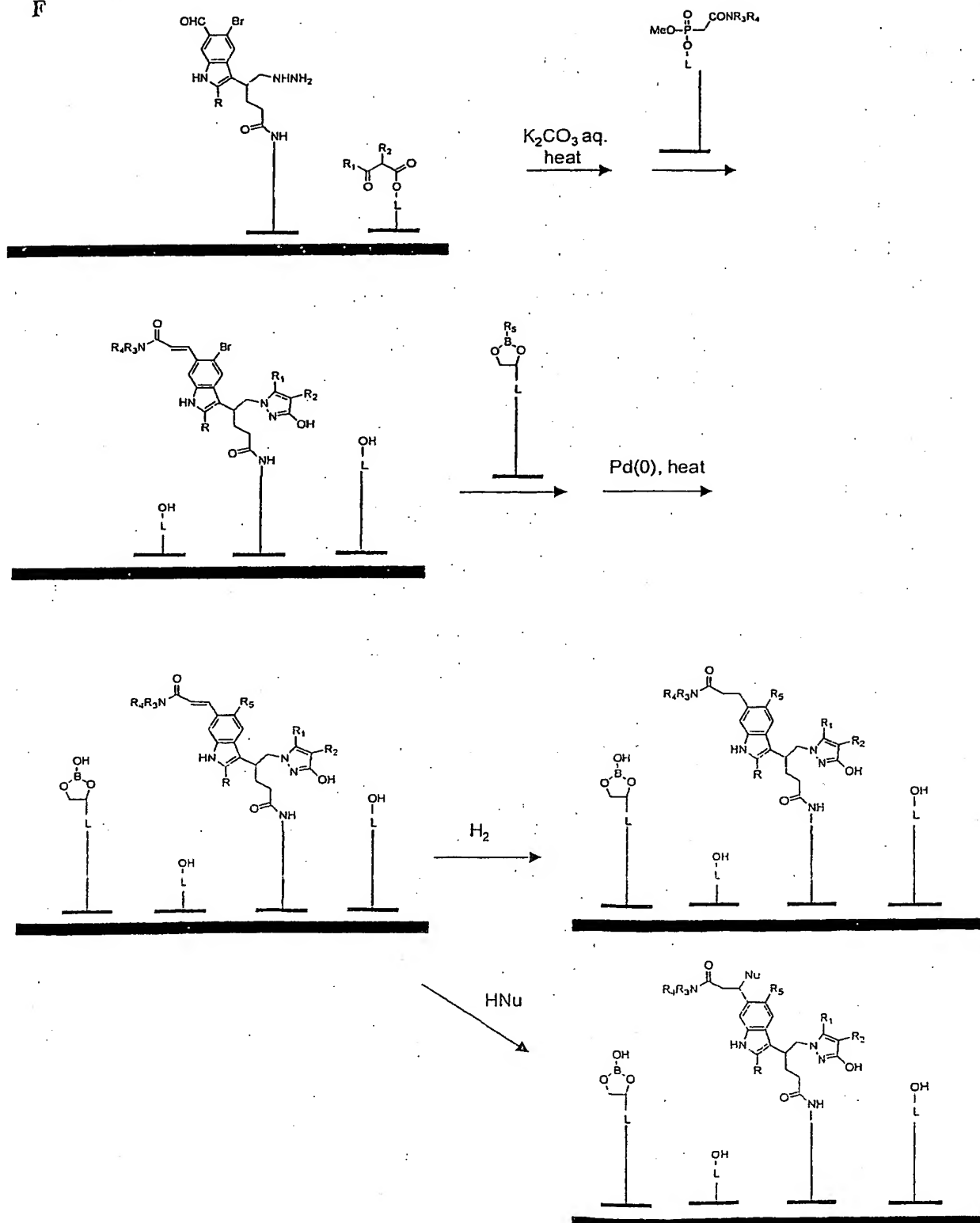
31/60

Fig. 18. Templated synthesis of non-linear molecules, examples.



32/60

F



33/60

Fig. 19. Templed synthesis in which the reaction step is performed under conditions where specific annealing of building block to template is inefficient.

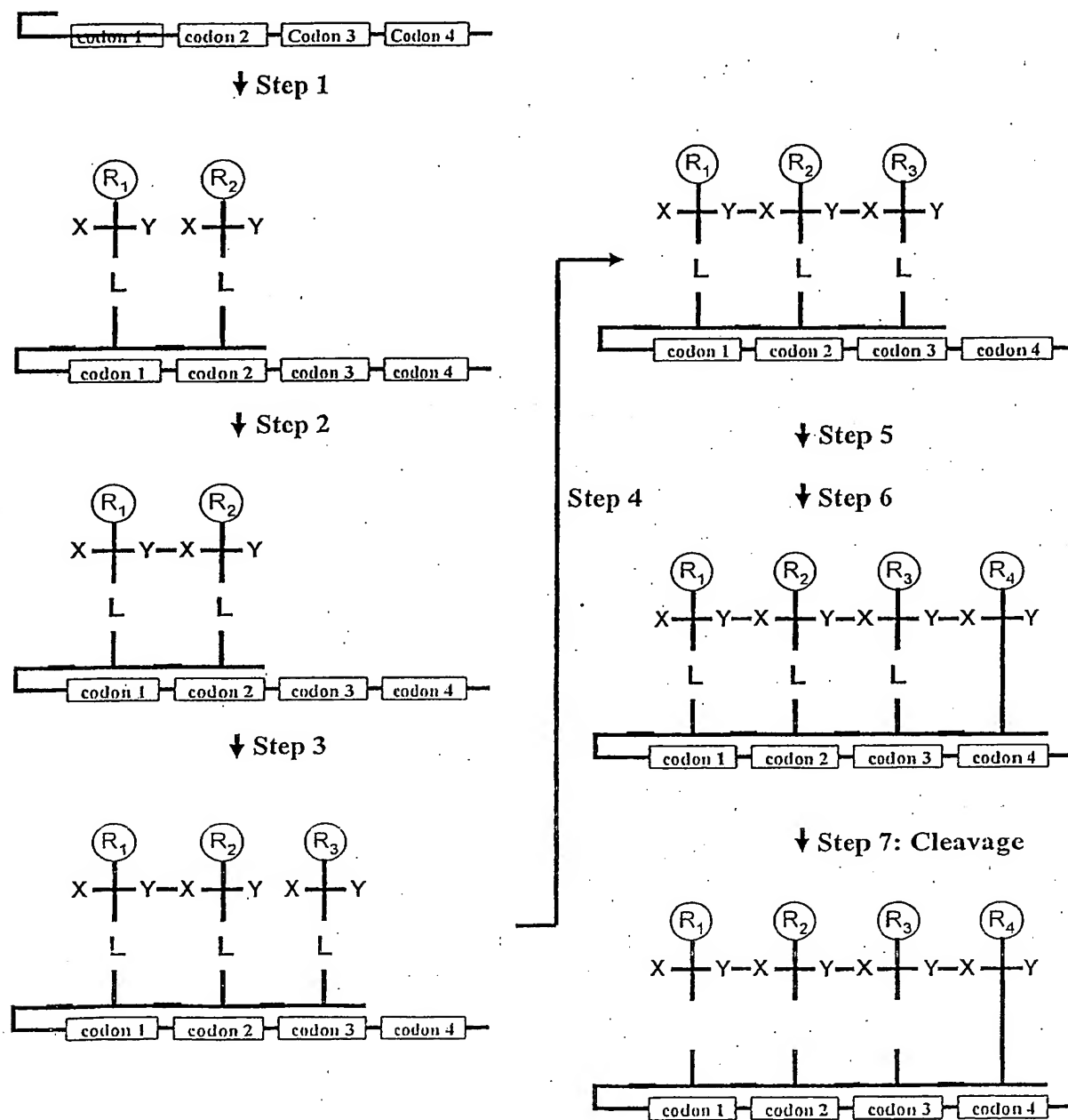
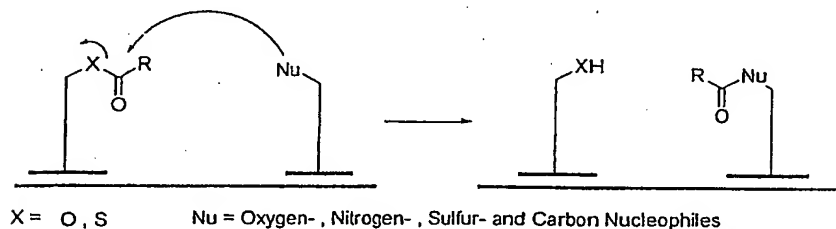
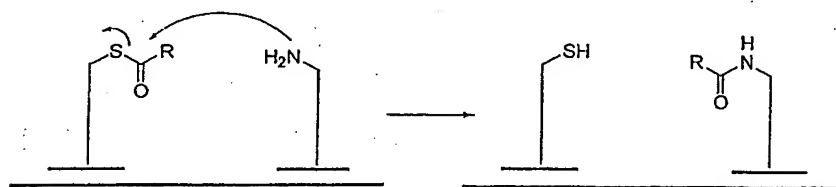
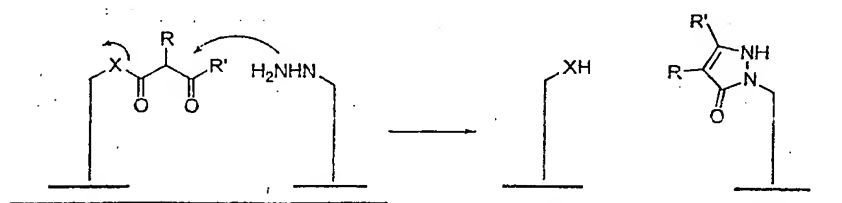
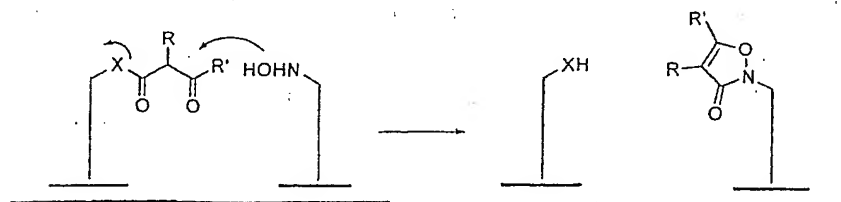
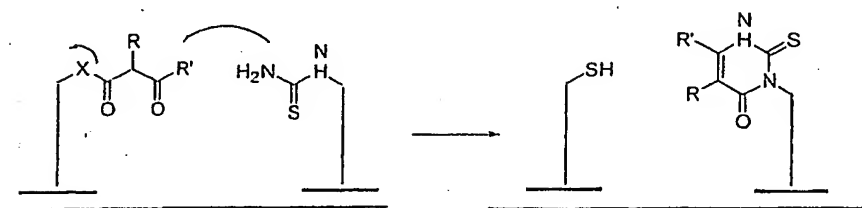
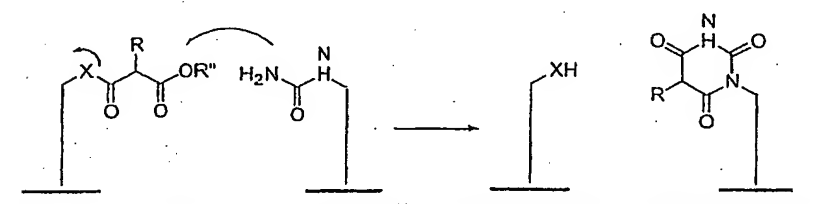
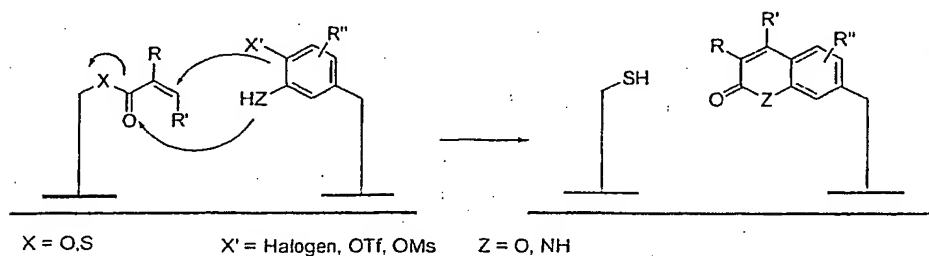
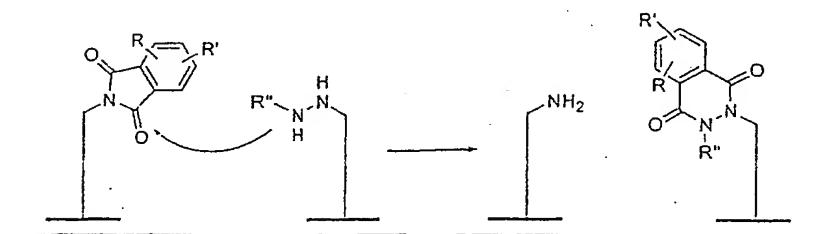


Fig. 20. Reaction types allowing simultaneous reaction and activation.**Nucleophilic substitution using activation of electrophiles****A. Acylating monomer building blocks - principle****B. Acylation**

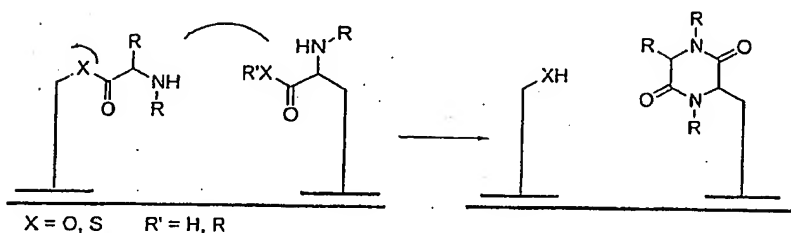
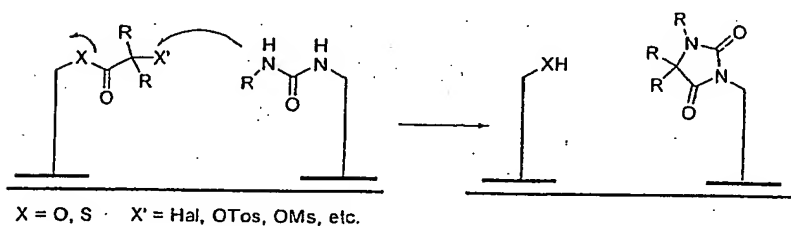
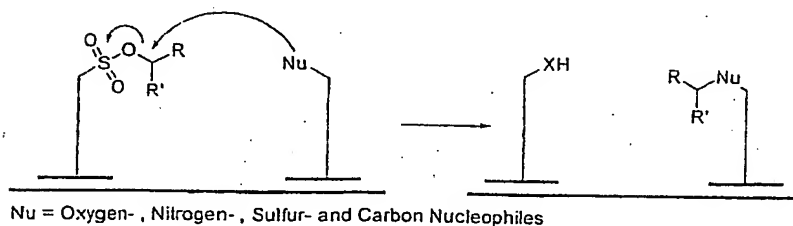
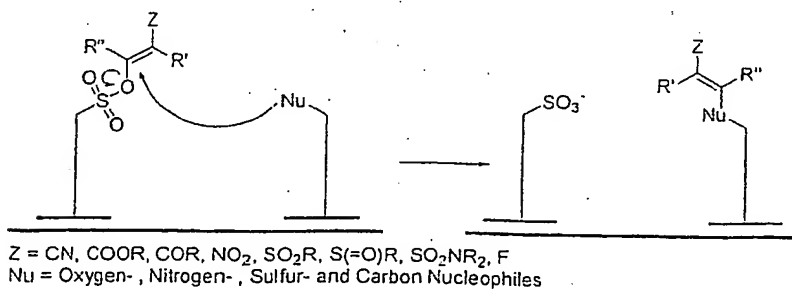
Amide formation by reaction of amines with activated esters

**C. Acylation**Pyrazolone formation by reaction of hydrazines with β -Ketoesters**D. Acylation**Isoxazolone formation by reaction of hydroxylamines with β -Ketoesters

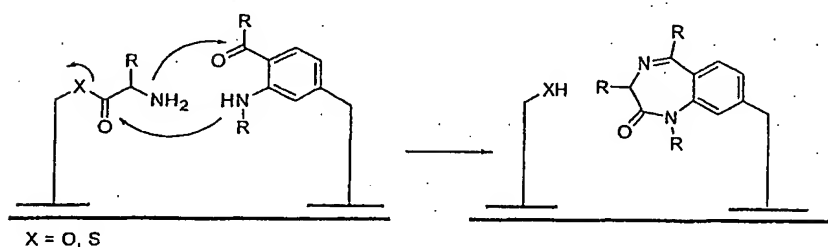
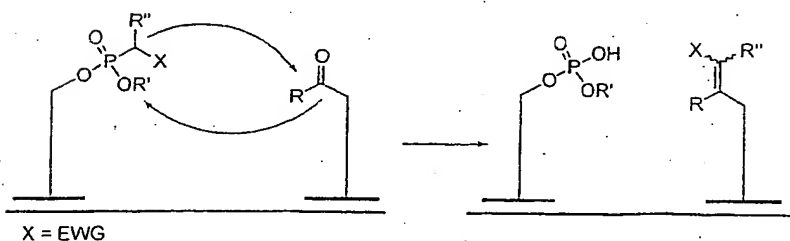
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E. Acylation**Pyrimidine formation by reaction of thioureas with β -Ketoesters****F. Acylation****Pyrimidine formation by reaction of ureas with Malonates****G. Acylation****Coumarine or quinolinon formation by a Heck reaction followed by a nucleophilic substitution****H. Acylation****Phthalhydrazide formation by reaction of Hydrazines and Phthalimides**

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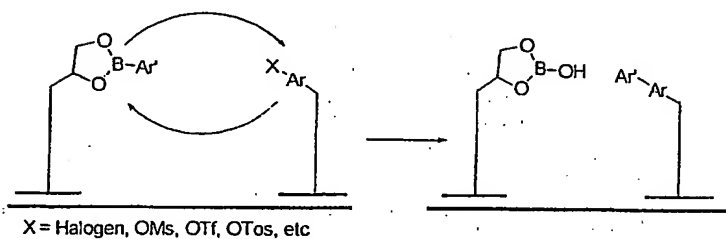
I. Acylation**Diketopiperazine formation by reaction of Amino Acid Esters****J. Acylation****Hydantoin formation by reaction of Urea and α -substituted Esters****K. Alkylating monomer building blocks - principle****Alkylated compounds by reaction of Sulfonates with Nucleophiles****L. Vinylating monomer building blocks - principle**

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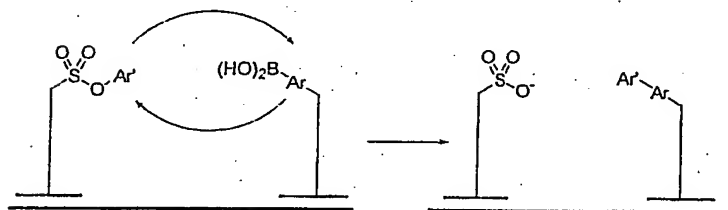
M. Heteroatom electrophiles**Disulfide formation by reaction of Pyridyl disulfide with Mercaptanes****N. Acylation****Benzodiazepinone formation by reaction of Amino Acid Esters and Amino Ketones****Addition to carbon-hetero multiple bonds****O. Wittig/Horner-Wittig-Emmons reagents****Substituted alkene formation by reaction of Phosphonates with Aldehydes or Ketones**

Transition metal catalysed reactions**P. Arylation**

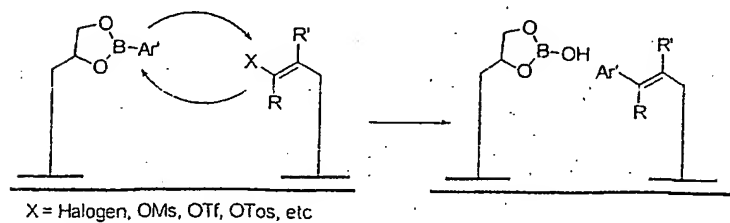
Biaryl formation by the reaction of Boronates with Aryls or Heteroaryls

**Q. Arylation**

Biaryl formation by the reaction of Boronates with Aryls or Heteroaryls

**R. Arylation**

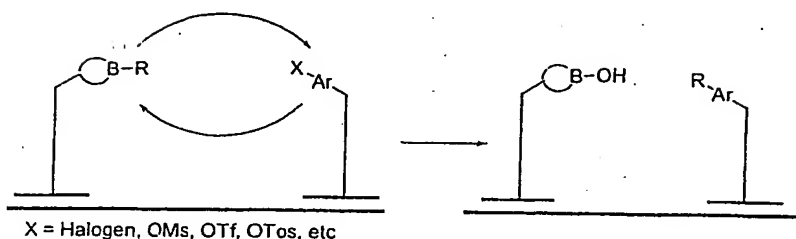
Vinylarene formation by the reaction of alkenes with Aryls or Heteroaryls



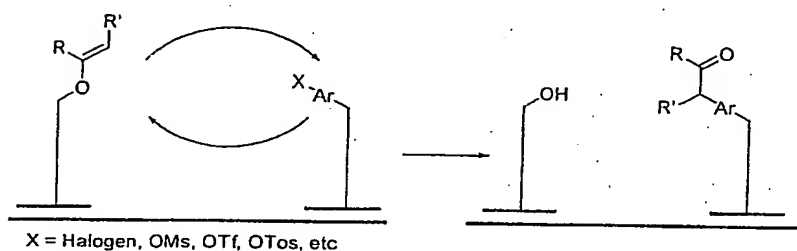
39/60

S. Alkylation

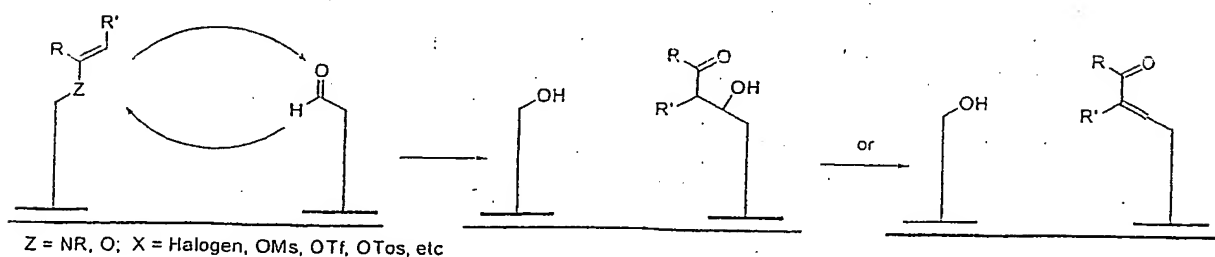
Alkylation of arenes/hetarens by the reaction with Alkyl boronates

**T. Alkylation**

Alkylation of arenes/hetarens by reaction with enolethers

**Nucleophilic substitution using activation of nucleophiles****U. Condensations**

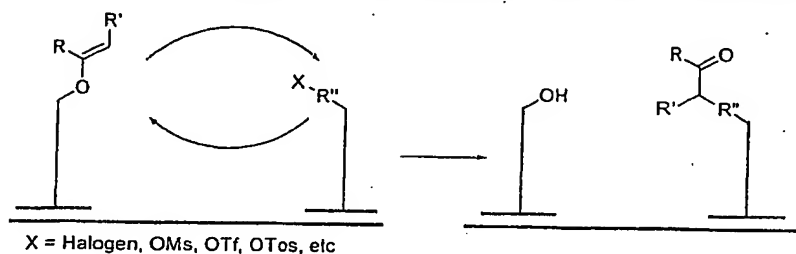
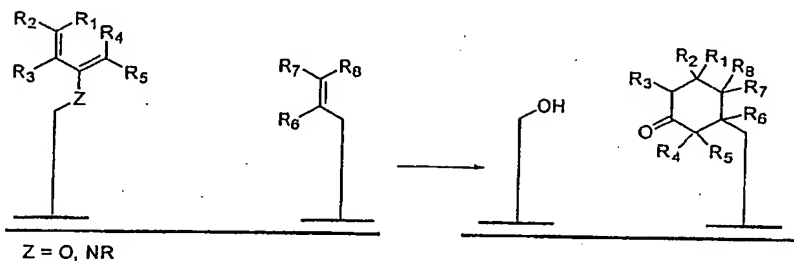
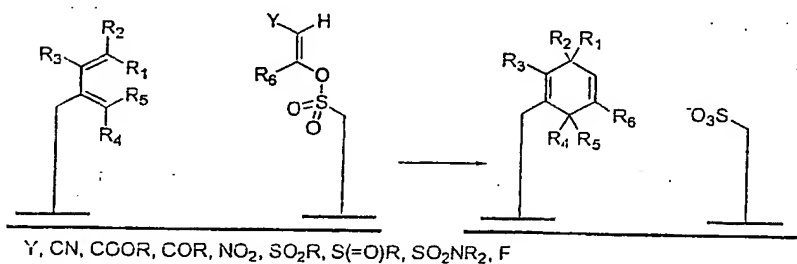
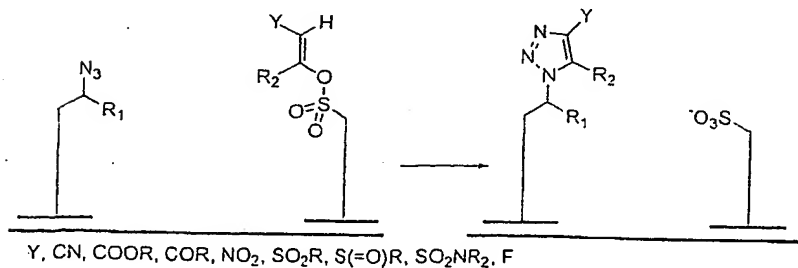
Alkylation of aldehydes with enolethers or enamines



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V. Alkylation

Alkylation of aliphatic halides or tosylates with enolethers or enamines

**Cycloadditions****W. [2+4] Cycloadditions****X. [2+4] Cycloadditions****Y. [3+2] Cycloadditions**

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Z. [3+2] Cycloadditions

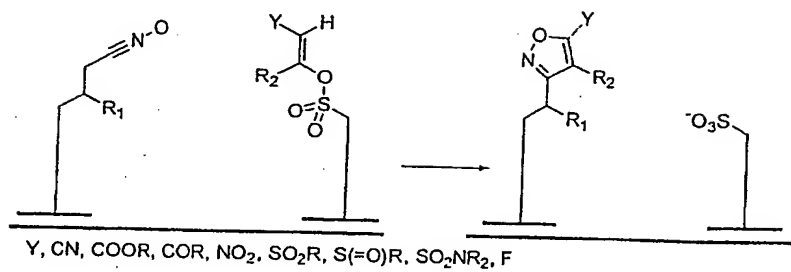
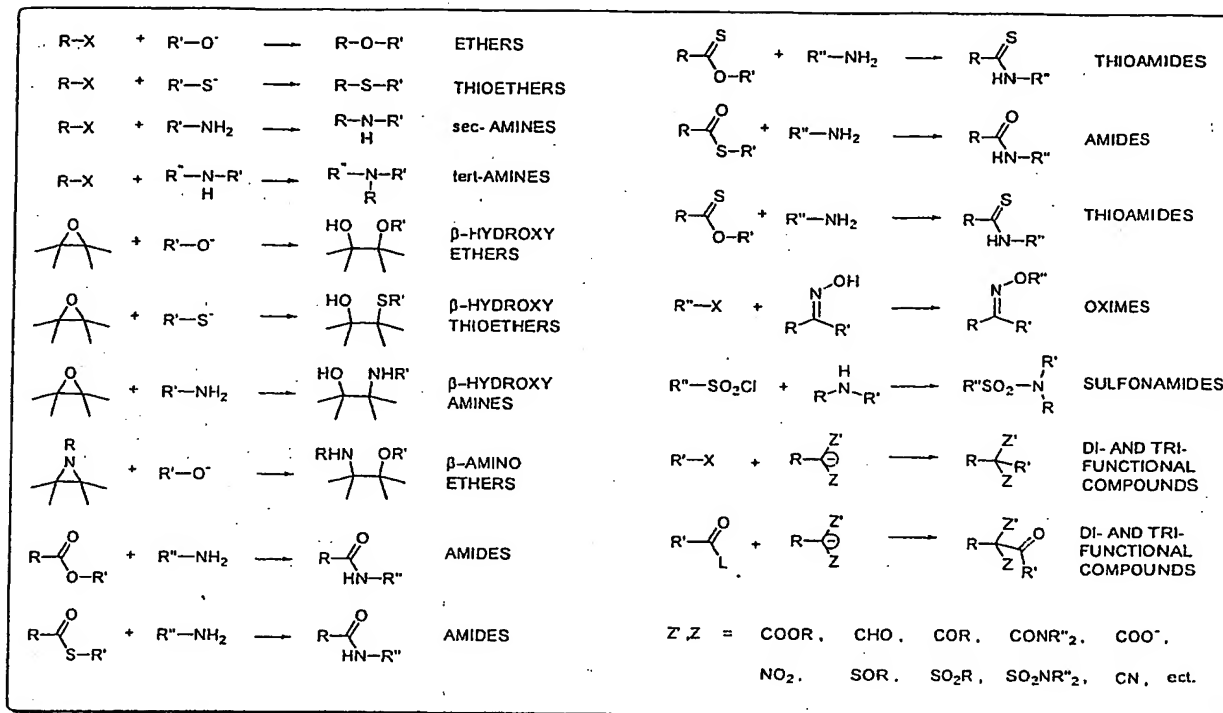
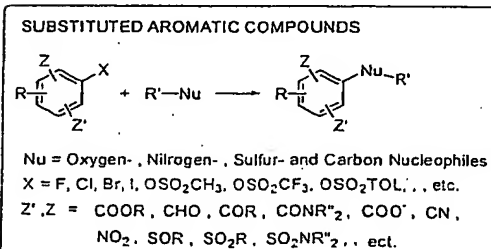


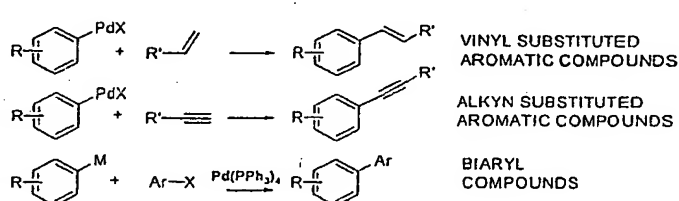
Fig. 21. Pairs of reactive groups X,Y and the resulting bond XY.
Nucleophilic substitution reaction



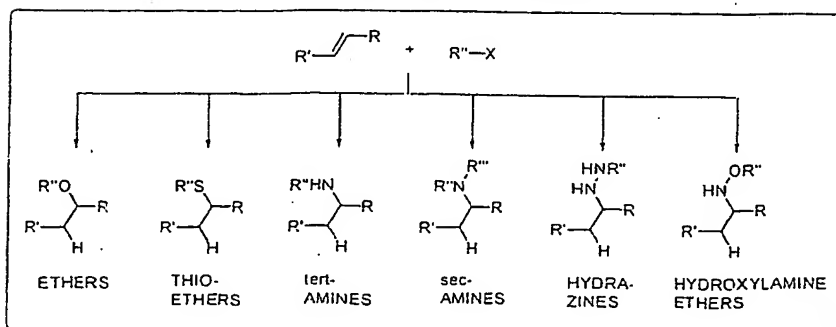
Aromatic nucleophilic substitution



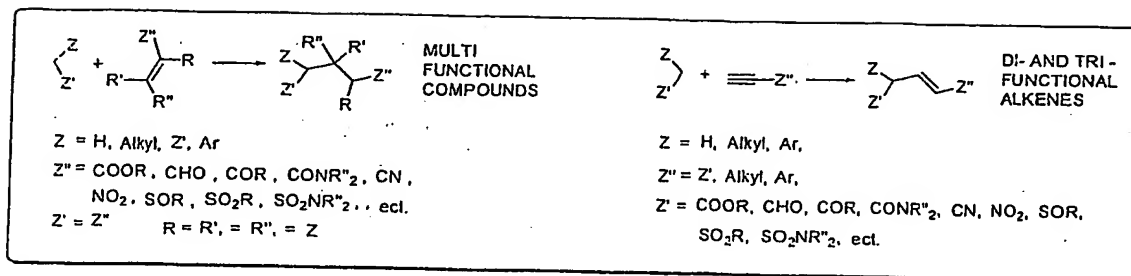
Transition metal catalysed reactions



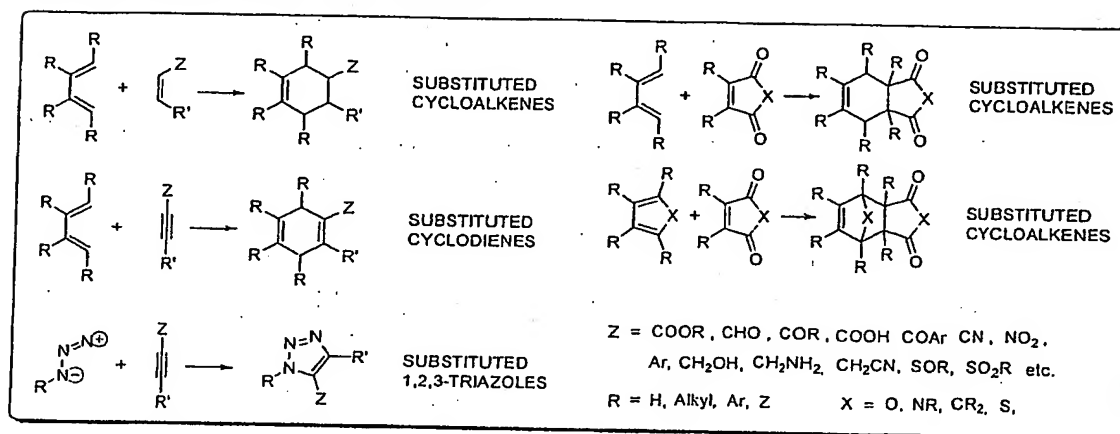
Addition to carbon-carbon multiplebonds



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Cycloaddition to multiple bounds



Addition to carbon-hetero multiple bonds

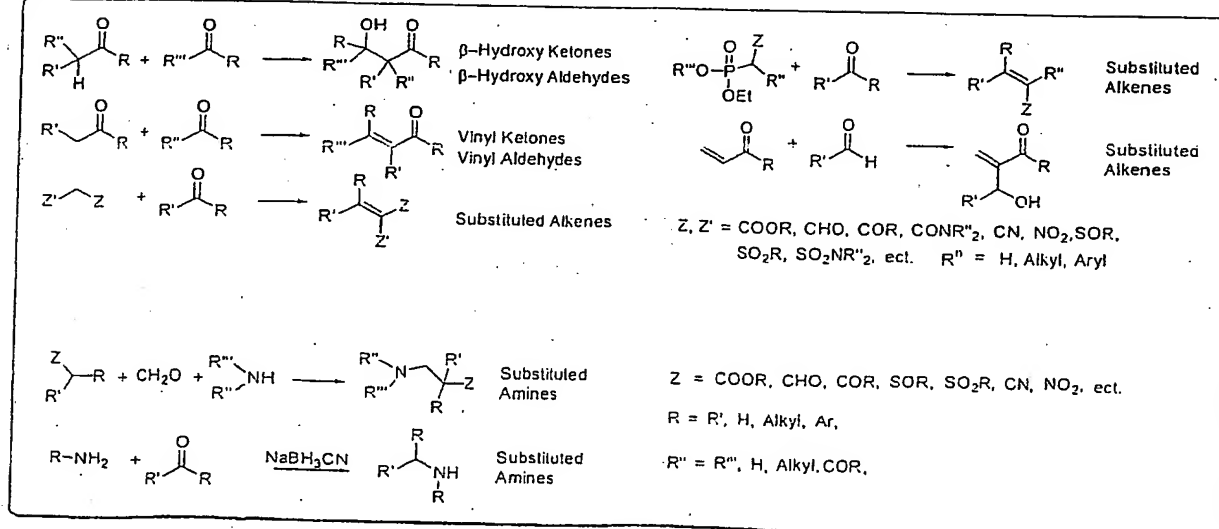
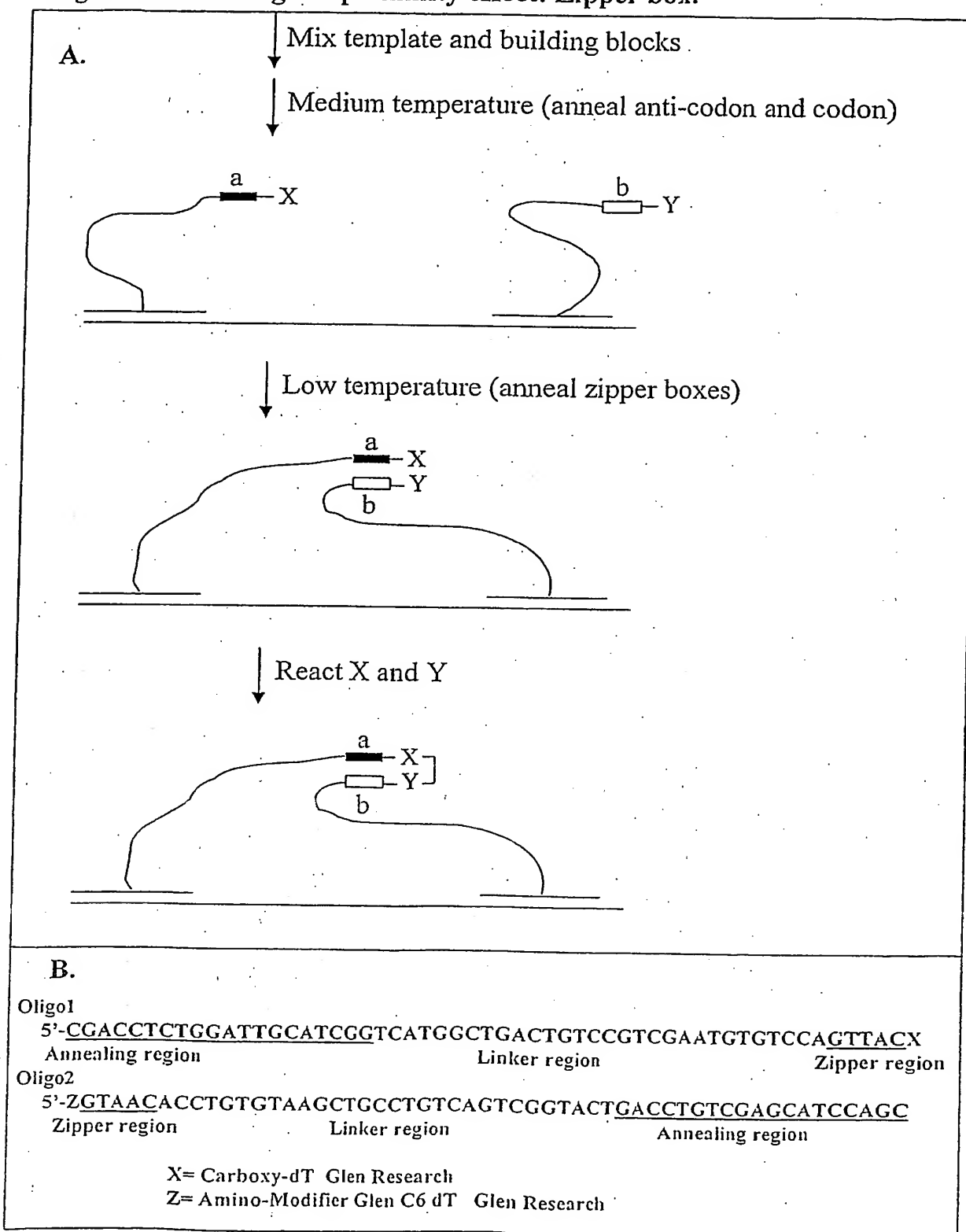
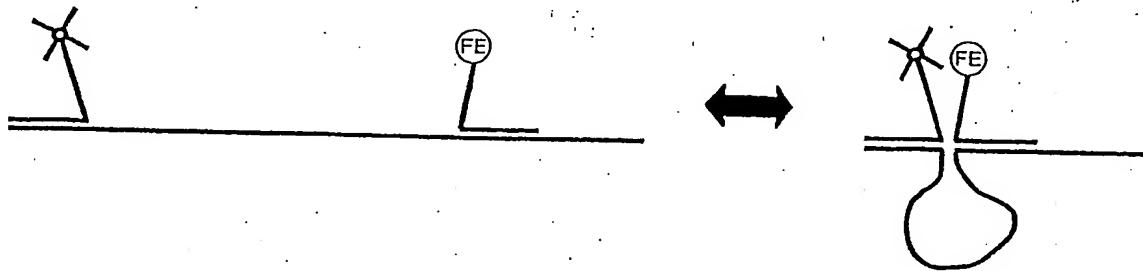


Fig. 22. Increasing the proximity effect: Zipper box.

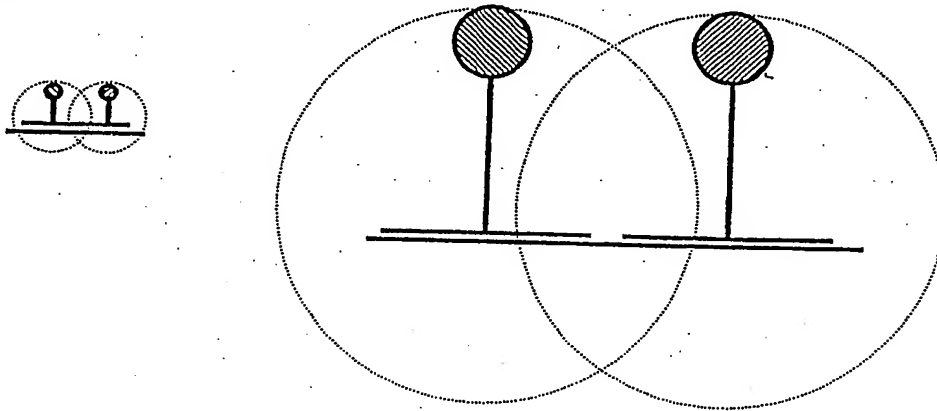
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Fig. 23. Increasing the proximity effect: Helix Stacking (A), Ligation (B), and (C) Rigid linkers

A. Double helix stacking.



B. Ligation.



C. Rigid linkers.

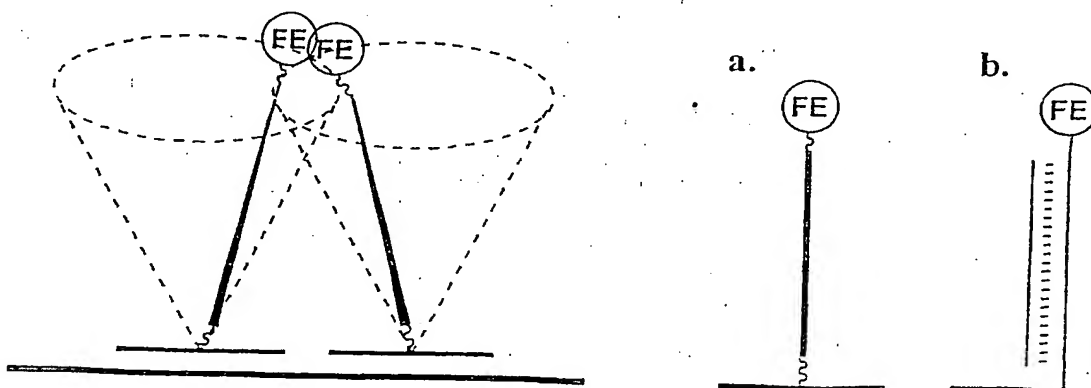
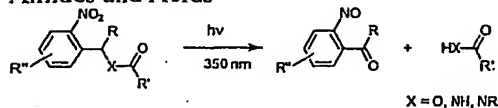


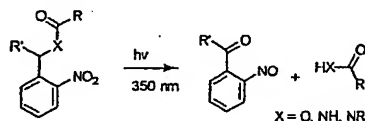
Fig. 24. Cleavable Linkers

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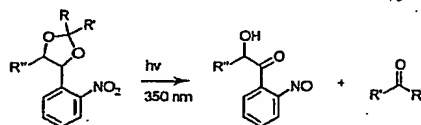
A. Linker for the formation of Ketones, Aldehydes, Amides and Acids



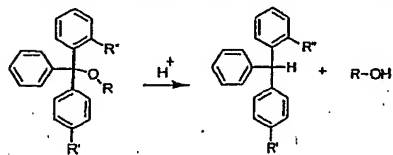
B. Linker for the formation of Ketones, Amides and Acids



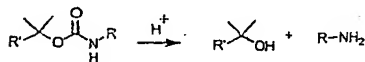
C. Linker for the formation of Aldehydes and Ketones



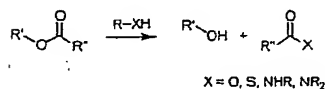
D. Linker for the formation of Alcohols and Acids



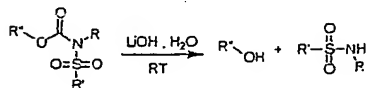
E. Linker for the formation of Amines and Alcohols



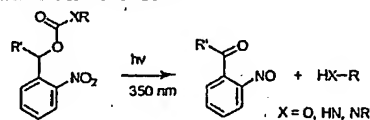
F. Linker for the formation of Esters, Thioesters, Amides and Alcohols



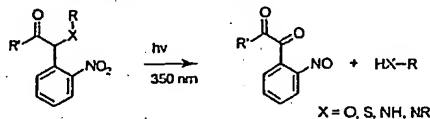
G. Linker for the formation of Sulfonamides and Alcohols



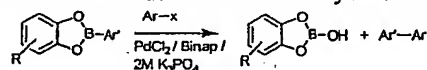
H. Linker for the formation of Ketones, Amines and Alcohols



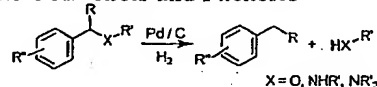
I. Linker for the formation of Ketones, Amines, Alcohols and Mercaptanes



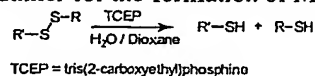
J. Linker for the formation of Biaryl and Bihetaryl



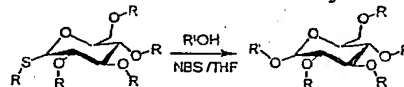
K. Linker for the formation of Benzyles, Amines, Anilins Alcohols and Phenoles



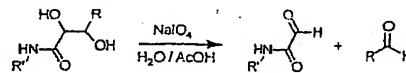
L. Linker for the formation of Mercaptanes



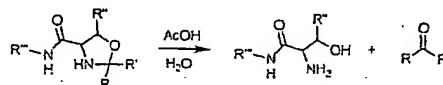
M. Linker for the formation of Glycosides



N. Linker for the formation of Aldehydes and Glyoxylamides

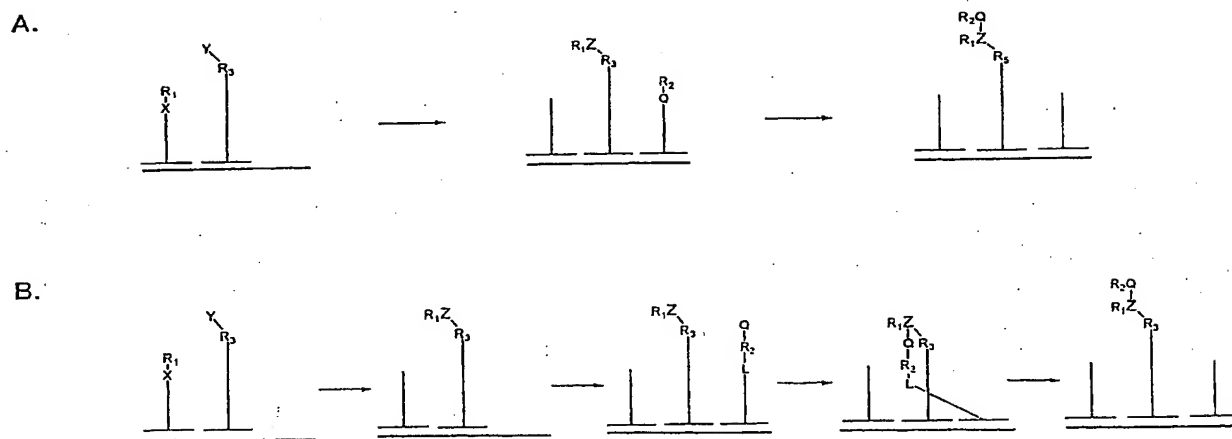


O. Linker for the formation of Aldehydes, Ketones And Aminoalcohols



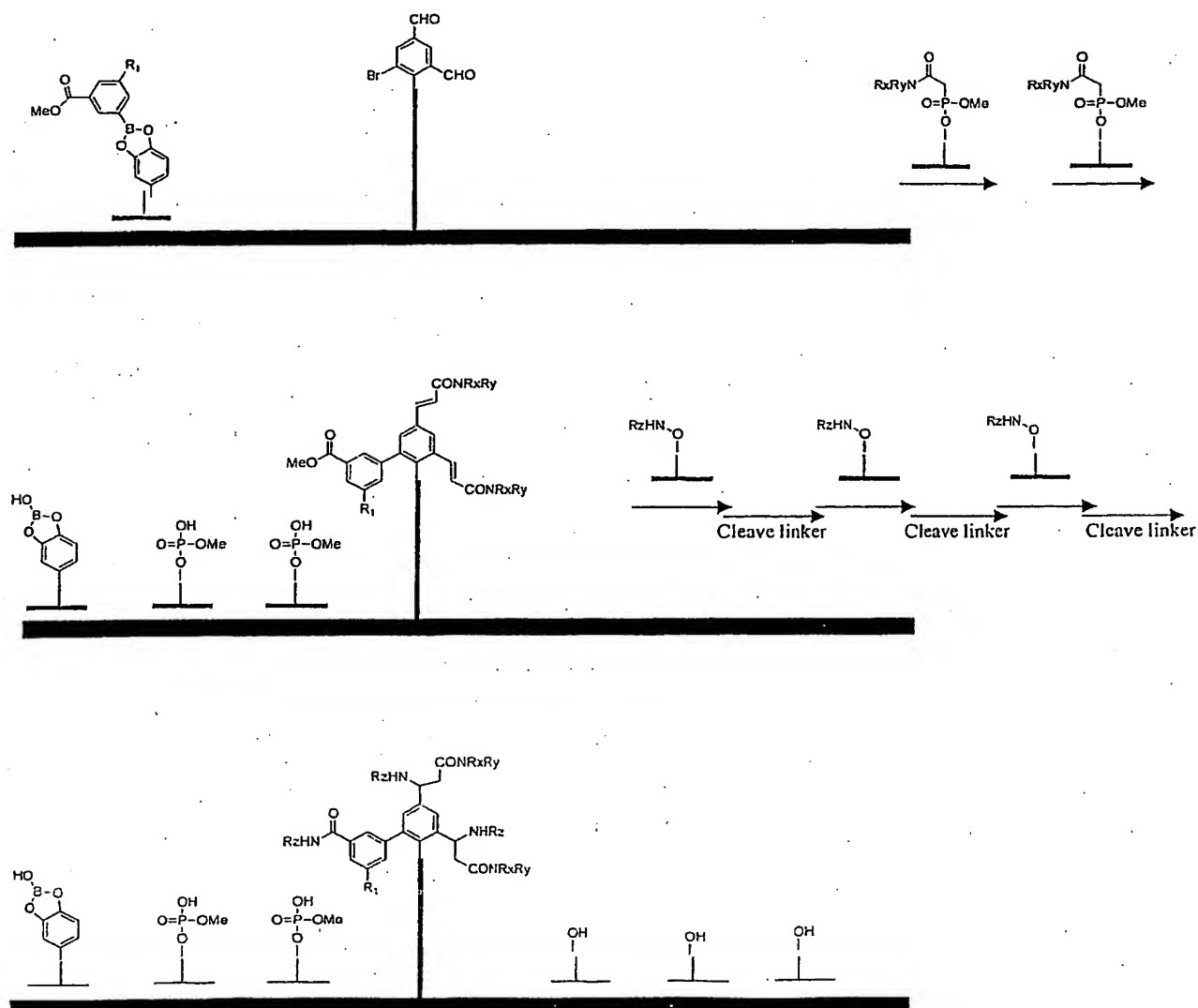
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Fig. 25. Templated synthesis by generating a new reactive group.



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Fig. 25, example 1. Generation of reactive groups in the first reaction round, followed by reaction of the generated reactive groups with introduced reactive groups.



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Fig. 26. Post-templating modification of templated molecule

A Rearrangement and cleavage in one step, eg:

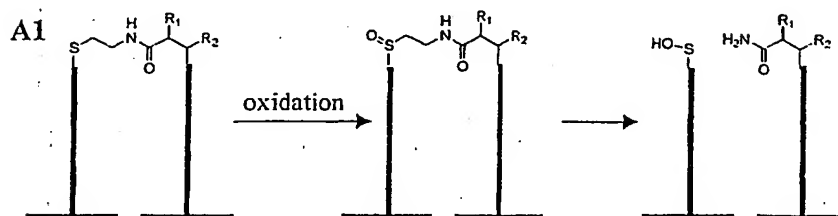
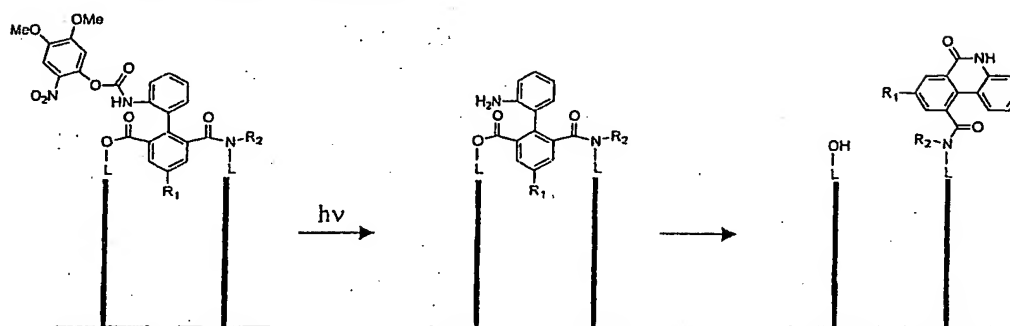
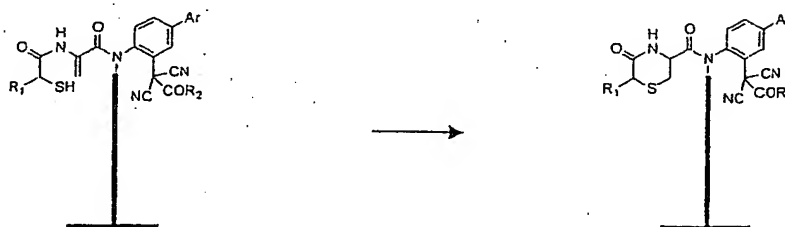
**A2**

Photo labile protecting group

**B Reaction of functional groups present in a templated molecule****B1 Intramolecular Michael addition:**

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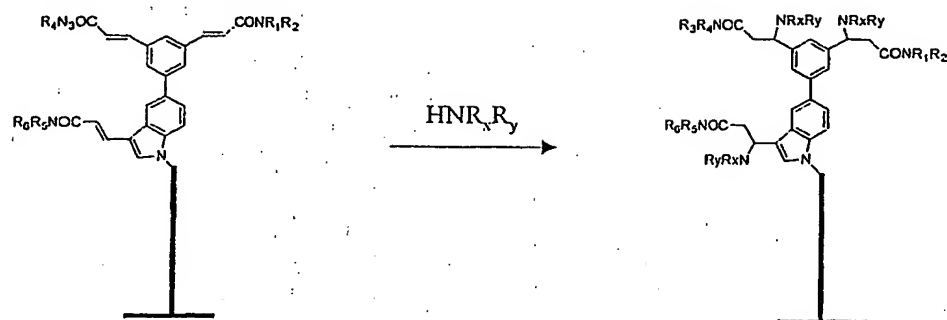
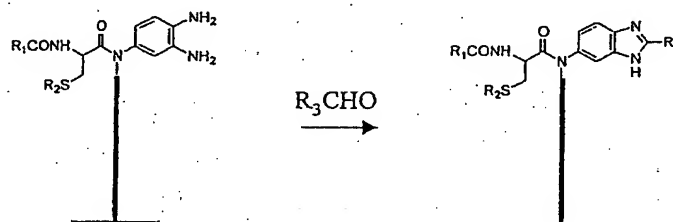
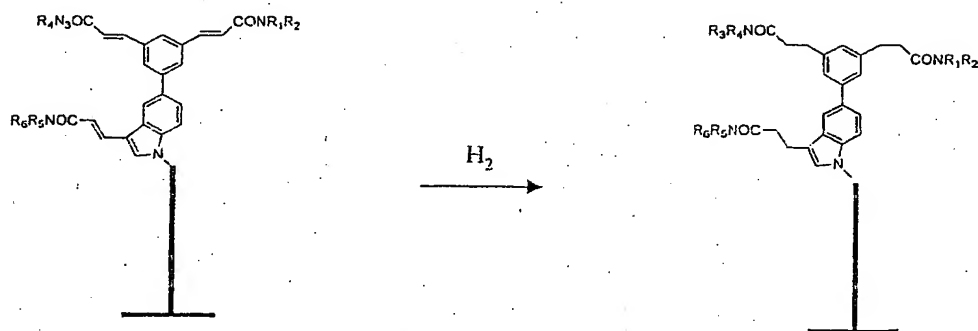
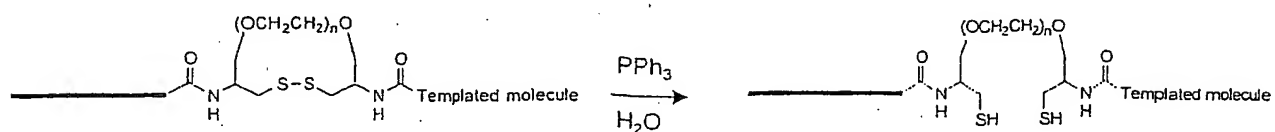
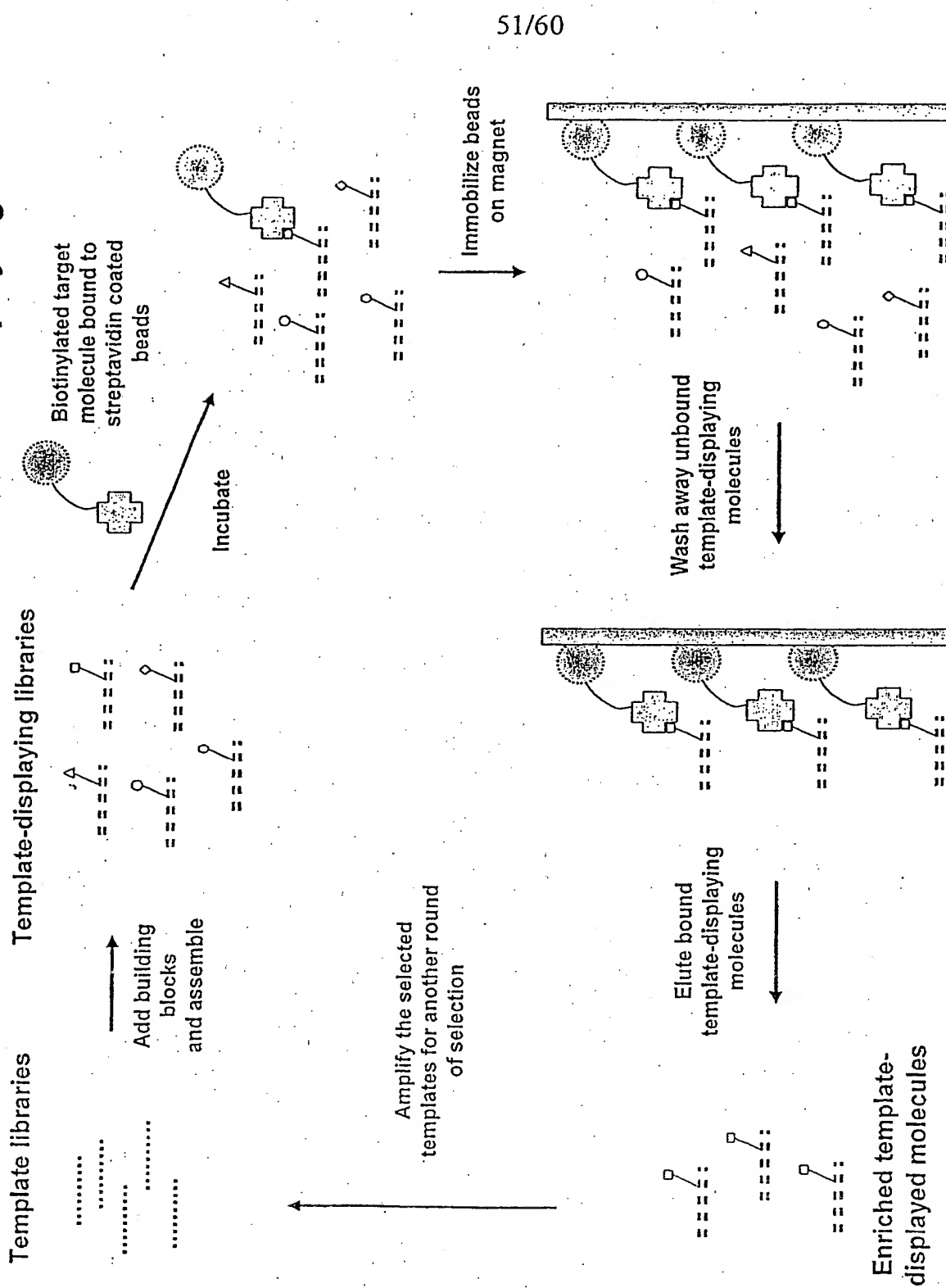
B2 Intermolecular Michael addition:**B3 Reaction of phenylenediamines and aldehydes to form benzimidazoles:****B4 Reduction of multiple bonds:****C Post templating modification of linker to extend the spacing between the template and the templated molecule.**

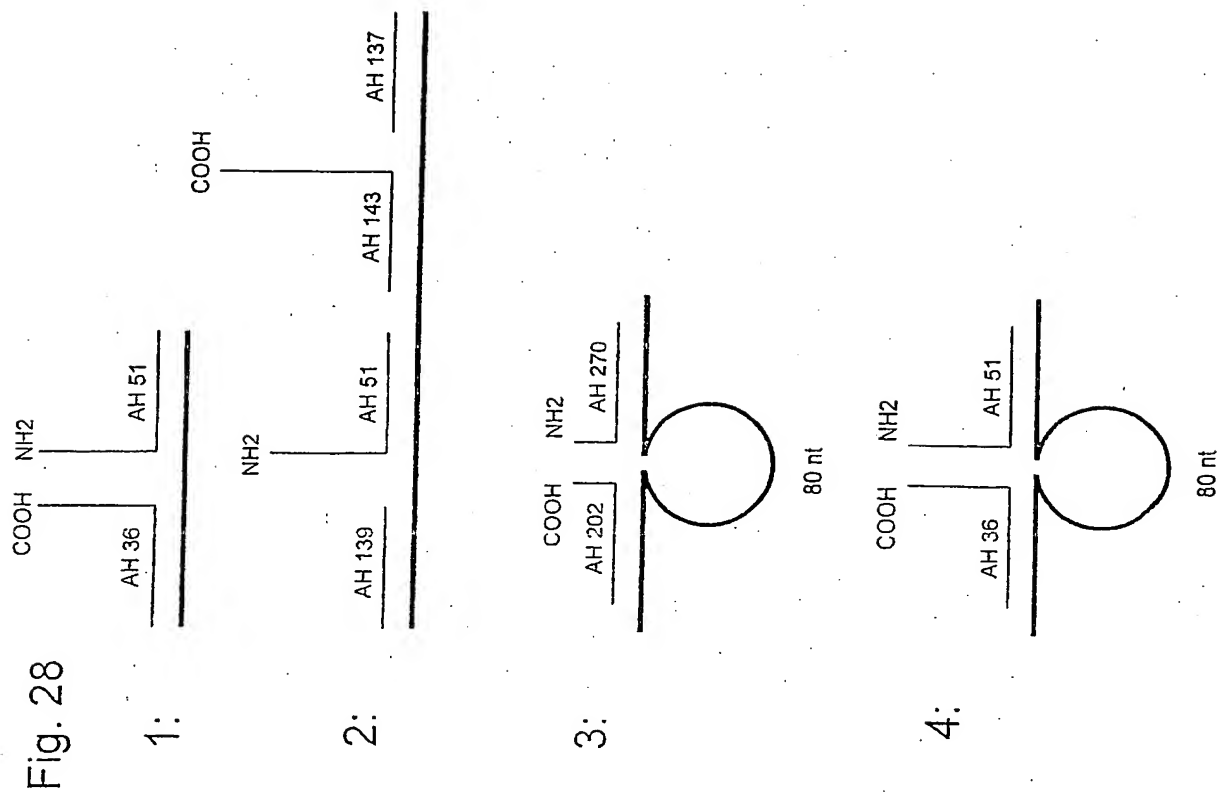
Figure 27.
A typical panning protocol for selection of template-displaying molecules



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Reaction efficiency



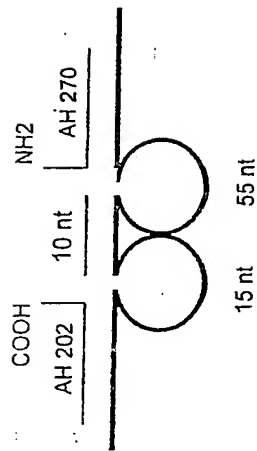
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Reaction efficiency

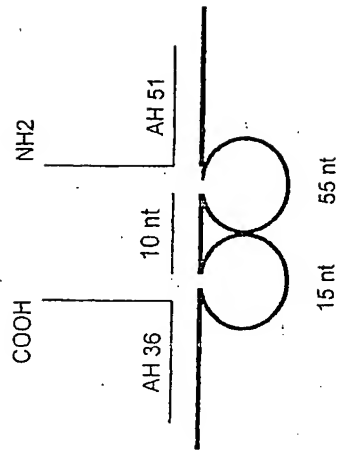
Fig. 28

(+)**++****-**

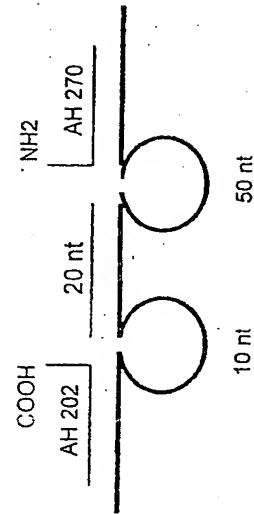
5:



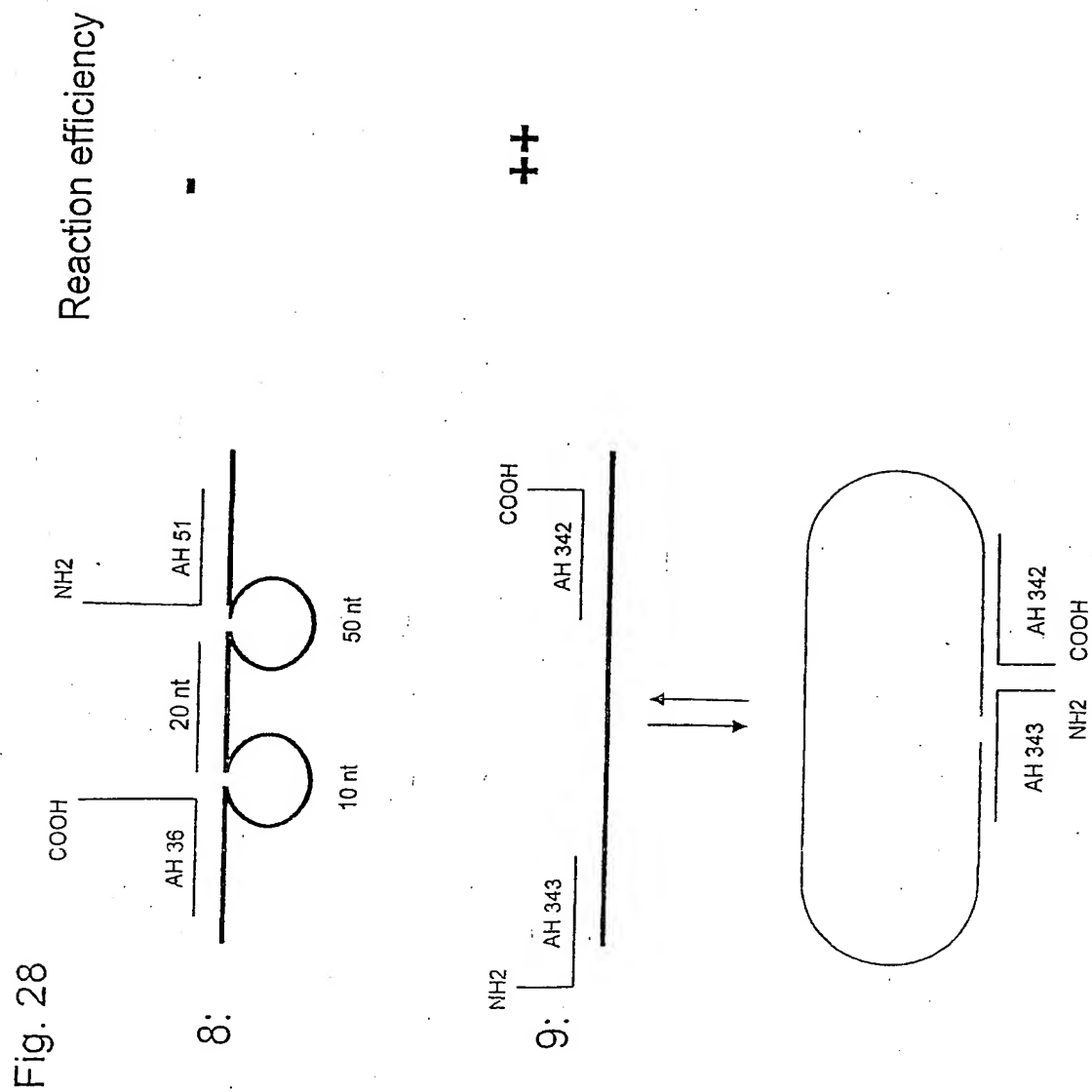
6:



7:



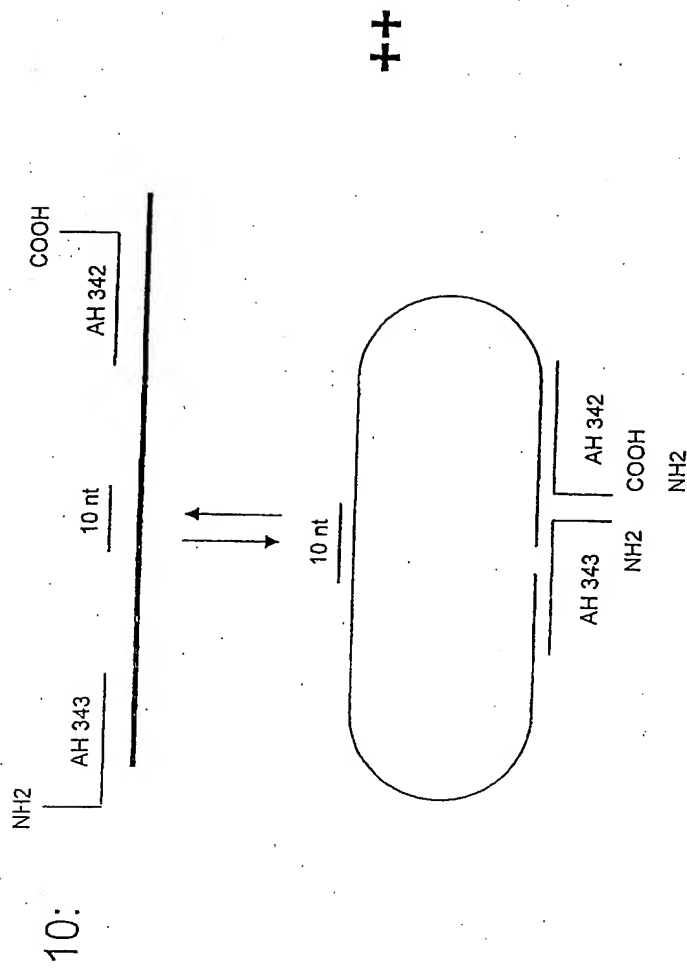
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Reaction efficiency

Fig. 28



Reaction efficiency

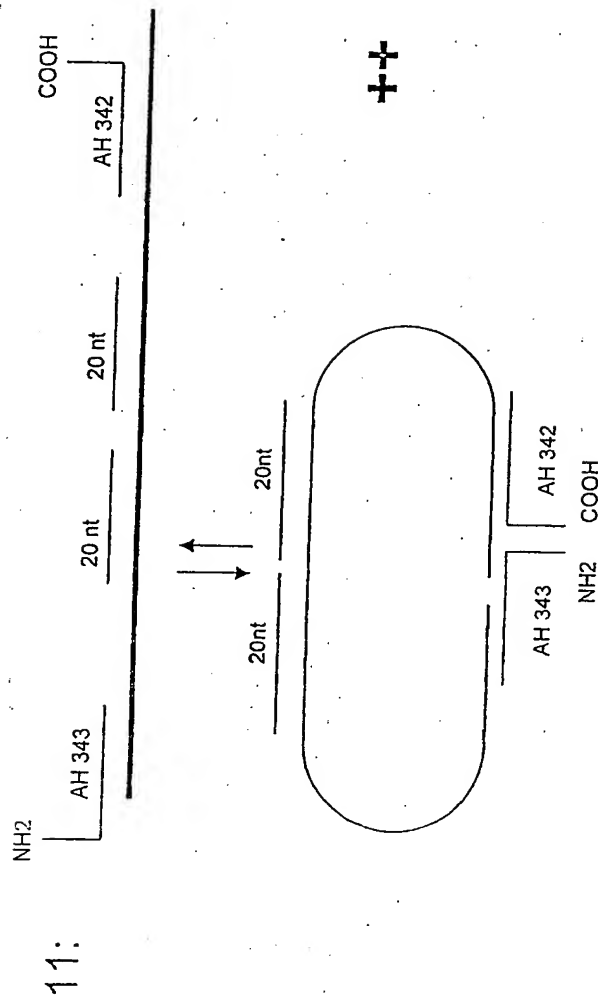


Fig. 28

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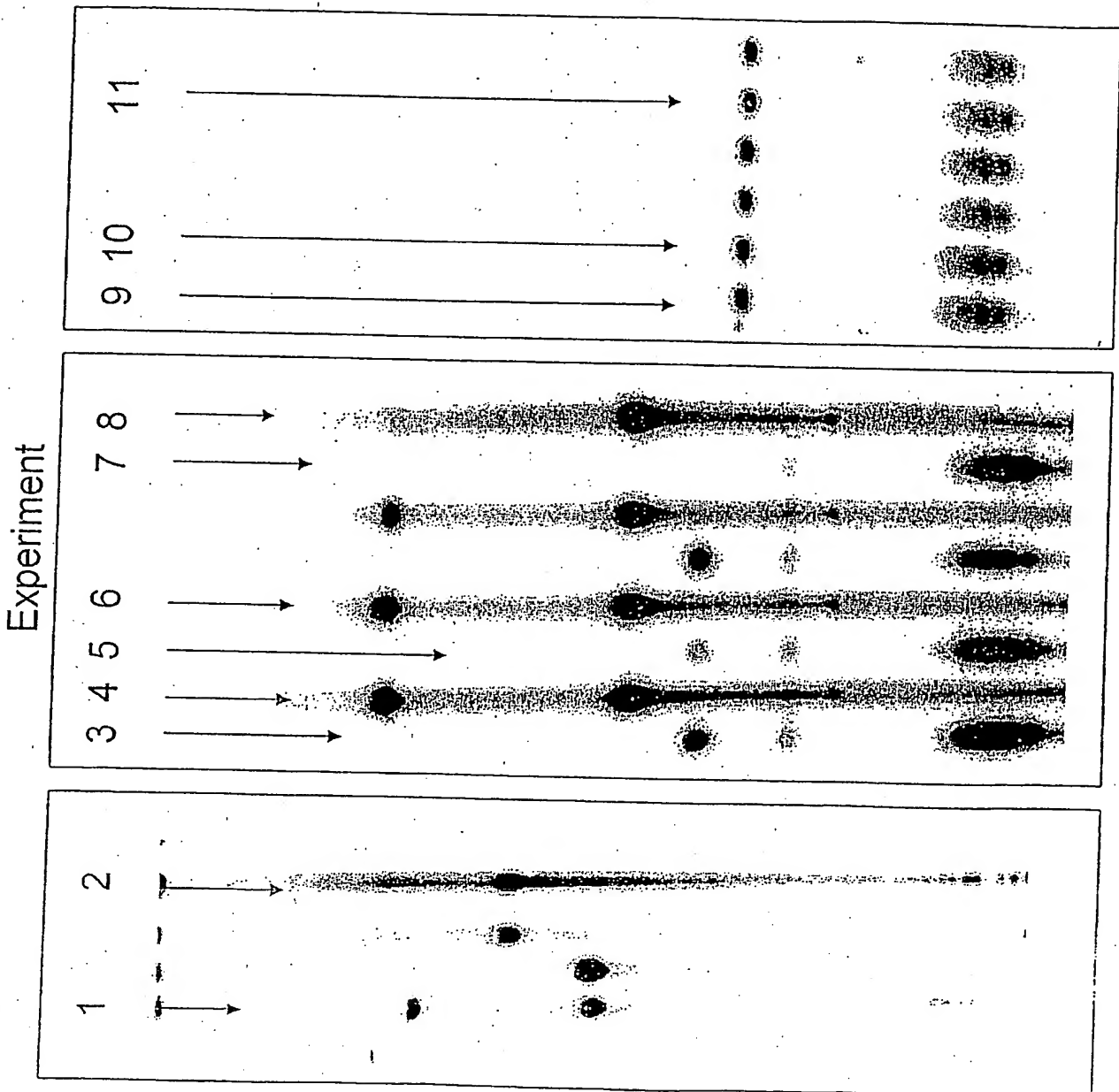


Fig. 29

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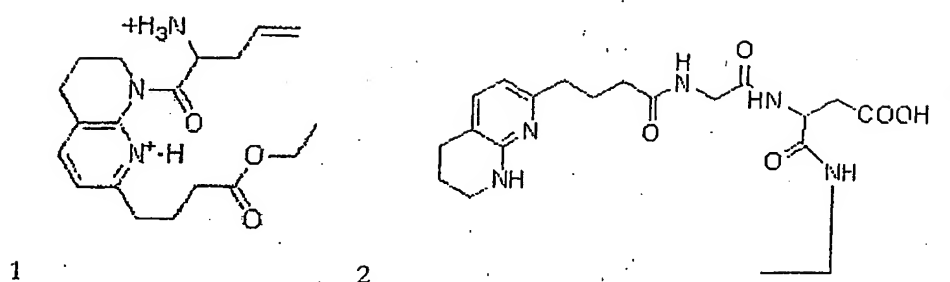


Figure 30. Structure 1 shows the Feuston 3 functional entity, which is needed together with Gly and Asp to create Feuston 5 structure 2, a ligand that binds to the $\alpha_v\beta_3$ integrin receptor (as described in press; Feuston BP et al. J Med Chem. 2002 Dec 19;45(26):5640-8)

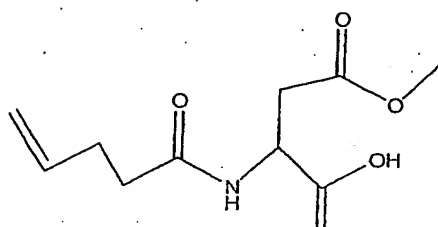


Figure 31. Structure of the pentenoyl protected aspartate functional entity used to load an amino modified scaffold oligo, to create the Feuston 5 ligand.

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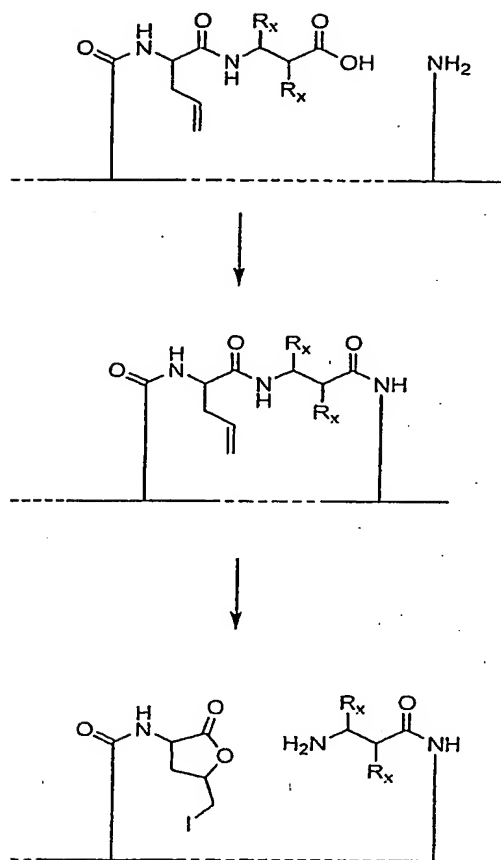


Figure 32. Allylglycine chemistry illustrated by structure showing cross-linked product as well as transferred product after cleavage by iodine.

1 2 3 4 5 6



Figure 33. An autoradiography showing the three transfers of β -Ala to an amino modified scaffold oligo, this scaffold oligo being radioactively labeled. Lanes 1, 3 and 5 shows cross-linked product between scaffold amine and functional entity β -Ala AG carboxylic acid for transfers 1, 2 and 3. Lanes 2, 4 and 6 shows cleaved product, i.e. scaffold carrying the transferred functional entity.

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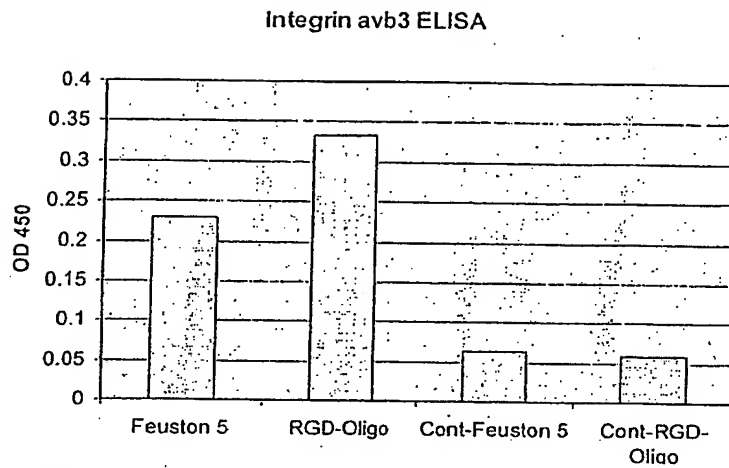


Figure 34 Result from the ELISA done on the feuston 5 ligand generated by sequential transfers to a scaffold oligo (first column). The controls are the RGD peptide, which is an Integrin ligand (second column;) loaded on a 20 mer oligo, and uncoated wells (no Integrin immobilized; third and fourth columns).